ENVIRONMENTAL REACTIVITY FOR BETTER OR WORSE

The impact of stress and reward on neurochemistry, affect and behavior across the psychosis continuum

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The impact of stress and reward on neurochemistry, affect and behavior across the psychosis continuum

DISSERTATION

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There is a crack in everything. It's how the light gets in.

- Leonard Cohen

Contents

Abbreviations

ALE	Activation likelihood estimation
BA	Brodmann area
BDNF	Brain-derived neurotrophic factor
BOLD	Blood ocygen leven-dependen (response)
BP_{ND}	Binding potential relative to non-displaceable radioligand
BPRS	Brief Psychiatric Rating Scale
COMT	Catechol-o-methyltransferase
CECA	Childhood experience of care and abuse
CAPE	Community assessment of psychich experiences
CDG	Calgary depression scale
CNC	Caudate nucleus
CS	Conditioned stimulus
$D_{2/3}$	Dopamine receptor subtype 2/3
DA	Dopamine
DAergic	Dopaminergic
DART	Dutch Adult Reading Test
DAT	Dopamine transporter
DSM-IV	Diagnostic and statistical manual of mental disorders (4^{th} ed.)
ESM	Experience sampling method
FIGS	Family interview for genetic studies
FWHM	Full-width half maximum
fMRI	Functional magnetic resonance imaging
GR	Glucocorticoid receptor
HPA	Hypothalamus-pituitary-adrenal axis
HV	Healthy volunteer
IFG	Inferior frontal gyrus
REL	First-degree relative of an individual with a psychotic disorder
LSRRM	Linear extension of the simplified reference region model
LTP	Long-term potentiation
MIST	Montreal imaging stress task
MNI	Montreal Neurological Institute
NA	Negative affect
NAPD	Non-affective psychotic disorder
PANSS	Positive and negative symptom scale
PA	Positive affect

PE	Prediction error/ Positive event
PET	Positron emission tomography
PFC	Prefrontal cortex
PSST	Probabilistic stimulus selection task
SANS	Scale for assessment of negative symptoms
SE	Self-esteem
SFG	Superior frontal gyrus
SRTM	Simplified reference tissue model
SS	Social stress
SZ	Schizophrenia
TAC	Time-activity curve
TC	Temporal cortex
UCS	Unconditioned stimulus
VOI	Volume of interest
ROI	Region of interest
VST	Ventral striatum
VTA	Ventral tegmental area
WASI	Wechsler abbreviated scale of intelligence
WRAT	Wide range achievement test
WTAR	Wechsler test of adult reading

Chapter 1

Introduction

On environment

Matter, energy, time and space came into being approximately 13.5 billion years ago, and have been coalescing into everything we see, know, and could think of ever since. That all makes up our environment.

All throughout our evolutionary history, during each human lifespan, and on a daily basis, we co-evolve with our environment by responding to its constant challenges to our status quo. The external negative factors – *stressors* – threaten our essential needs, resulting in *stress* – the internal state of activation of resources in order to fight or flight from the stressors. For instance, psychosocial stressors such as criticism or difficult tasks under time pressure have a particularly noxious effect on social and psychological wellbeing (1), motivating us to mitigate them. On the other hand, the positive environmental factors – *rewards* – satisfy some need, such as food, sex, social approval, or money, and therefore prompt behavior directed at obtaining them.

In other words, we continuously respond to our environment simply because it incentivizes us to do so. The quality and quantity of adaptive responsiveness to the environmental stresses and rewards determines how successful we are in it. In fact, how well adjusted one is to the current environment is, arguably, an essential metric of mental health.

On psychosis

Psychotic disorders, including schizofrenia and other non-affective psychoses, are characterized by impaired ability to recognize and relate to one's environment. Specifically, the positive symptoms of psychosis form by responding to an altered internal or external environment, such as hallucinations, delusions and paranoia (2). For instance, a news story that is trivial to most people might stand out to a patient to a degree that an entire web of extraordinary ideas form around it, perceived to be just as real as anything else, as in the case of delusions.

Meanwhile, negative symptoms stem from diminished tendency to engage with the environment, giving rise to apathy and demotivation (3). Contrarily to the news story, information that is important to most people- invitation to join a dinner party- might go unnoticed or provoke little desire in a patient with psychosis.

Consequently, much research has been devoted to the role of impaired ability to respond to the environmental demands in precipitating psychotic disorder. Hypersensitivity to stress has been shown to act as a potent catalyst in the onset and exacerbation of psychosis (4, 5), especially its positive symptoms (6). The scale of the psychotogenic effects of psychosocial stress appears to be directly related to their intensity and duration; chronic, severe stress induced by discrimination and childhood trauma has been shown to substantially increase the odds of developing the psychotic disorder (7, 8); mild, acute stressors, such as the company of strangers or mental effort

under pressure, have been shown to provoke equally mild and transient symptoms of psychosis (9).

At the same time, a separate line of research implicates diminished capacity to generate reward-oriented behavior in motivational deficits underlying the negative symptoms (10, 11). Despite the mounting evidence linking altered interactions with stresses and rewards with the symptoms of psychosis, the exact mechanisms rendering one vulnerable to these environmental factors remain poorly understood, and will therefore be closely examined in this dissertation.

On reactivity

The internal arousal intended to detect, sense, and respond to the environmental triggers is referred to as reactivity, and typically has a behavioral, physiological, and affective dimension.

Behavioral reactivity

Much daily behavior is controlled by stresses and rewards, and the capacity to modulate behavior in proportion to the kind and scale of the incentive cue has been found to differentiate between healthy state and psychosis (12), opening promising research avenues.

Inquiries into the reactivity to stress and reward have been relying on experimental tasks designed to approximate real-life stressful or rewarding circumstances, while isolating their essential properties from other external factors. This way, psychosocial stress has been induced in the laboratory using the Montreal Imaging Stress Task (MIST; (13), a mental arithmetic task under time pressure and negative evaluation, and Cyberball (14), a task simulating ostracism by gradually excluding the participant from an online ball game. Both manipulations have been found to increase arousal and negative affect in all participants(1, 13), but also induce paranoid ideation in paranoia-prone individuals (9, 15).

Reward, on the other hand, has been successfully examined using associative learning, particularly probabilistic reinforcement learning tasks where rewards are used to guide behavior (for a review see (16)). Here, the participant is presented with a choice of two digital stimuli, and required to select the better one using feedback, typically money, provided upon each choice. The better stimulus is reinforced more often, while its alternative is less advantageous. The main outcome measure is the accuracy in reinforcement learning – proportion of choices of the more frequently reinforced stimulus (17). In a series of experiments, the group of Gold frequently replicated deficiencies in reinforcement learning in patients with psychosis relative to controls (18, 19). Importantly, the constituents of this impairment, diminished sensitivity to rewards

and ability to modulate behavior as a function of rewards, were associated with the severity of the negative symptoms of psychosis (10, 11).

Physiological reactivity

Physiological reactivity to stress and reward is thought to be modulated by the brain, predominantly via the dopaminergic neurotransmission (20, 21). Animal studies have shown stress-induced dopamine (DA) release in the stress network: the medial prefrontal cortex (mPFC), nucleus accumbens (Nacc), putamen, and caudate nucleus (22, 23), with recent work proposing a reciprocal relationship between these hubs (24, 25). Importantly, they have also been shown to be exquisitely sensitive to rewards, signaling their presence and delivery by increased firing of the DA neurons (26, 27)

Dopamine has long been proposed to act as a neurochemical "label" that converts neutral stimuli into salient cues for action (20). In this framework, firing of the DA neurons in response to a stressor turns it into an experience that is emotionally relevant enough to trigger fight or flight behavior: arousal, selective attention, increased heart rate and sweating, in the cost of other functions (28). When DA is released in the presence of rewards, on the other hand, they become meaningful enough to prompt approach behavior: appetite, attention, effort, pleasure (29). Kapur famously applied this notion to explain the formation of positive and negative symptoms of psychosis (30). In this widely-accepted view, excessive (random or stress-induced) release of DA turns insignificant stimuli into motivational cues, while failing to detect the truly meaningful ones (30, 31). This explains why a trivial event, for example a news segment about computer technology, could lead a patient to believe to have a chip implanted into the brain, and result in unusual behavior, such as wearing a head-protective gear to avoid broadcasting the thoughts. Meanwhile, the potential rewards go undetected, resulting in the failure to engage in conversations or hobbies.

Advances in molecular neuroimaging enable non-invasive measurements of dopamine $D_{2/3}$ activity during the exposure to stress (with the MIST)(32, 33), or reward learning (via probabilistic reward task)(34), during Positron Emission Tomography (PET). Using the dopamine radiotracer ^{11}C-Raclopride, previous studies revealed significant stress-induced DA activity in the striatum of healthy individuals (28, 32). Importantly, this effect was found to be significantly potentiated in individuals with psychosis, and interpreted as the mechanisms of psychotogenic hyper-responsiveness to stress (35, 36).

While there is no shortage of findings of reward-related striatal DA activity in the general population (37-39), a comparative investigation in individuals with psychosis remains to be carried out.

High-affinity radiotracers ^{18}F-fallypride and ^{11}C-FLB457 designed to detect DA release in areas with much lower $D_{2/3}$ receptor densities, particularly the prefrontal cortex (PFC)(34), reliably indicated DA activity in response to both stress (40, 41) and re-

ward (42) in healthy individuals. Analogous findings are much more scare in patients with psychosis, however, with only a single report of blunted stress-induced DA release in the ventromedial prefrontal cortex (vmPFC) in those at familial risk for psychosis (43). Reward-induced prefrontal DA activity has, to date, not been investigated experimentally in the entire psychosis continuum. Chapter 2 will begin to fill this gap by examining both striatal and prefrontal DA reactivity to reward in individuals at genetic risk for psychosis and healthy controls.

Taken together, the abovementioned studies provide an intriguing, albeit initial and often sparse, evidence for DAergic basis for altered stress and reward reactivity in psychosis. One caveat in interpreting the available findings is, however, that the highly controlled laboratory settings are specializing in administration of the isolated forms of stressors and rewards, using instruments designed to capture a narrow range of their effects. While this approach allows for a systematic look into the fundamentals of the objective environmental reactivity, the nuances of the complex subjective response to the entangled environmental demands of the real world remain obscured.

Affective reactivity

In a trade-off between analytical and practical perspective, the experience sampling method (ESM) has been shown to be sensitive to the subtle affective fluctuations, yet robust to the complexities of the stressors and rewards presented by the everyday life (Delespaul, 1995)(44). ESM is a structured diary technique collecting multiple assessments per day for the duration of one week. Each assessment samples a random moment in the real life of the participants, prompting them to appraise their current mood, psychopathology, company and activities, as well as past and upcoming events (45). Using this method, stress has been linked to increased negative affect in patients with psychosis relative to controls (5). Importantly, paranoia has been associated with this exaggerated affective reactivity to stress (46), and shown to be modulated by exposure to childhood trauma (47), thus outlining the elements and trajectory of the psychotogenic reactivity to stress. The missing link between the objective and subjective reactivity to stress was provided by Hernaus and colleagues (2014) in a combined PET-ESM study that revealed an association between PFC DA activity under stress and psychotic reactivity to daily-life stress in individuals with genetic risk for psychosis (48). An outstanding issue is the unknown impact of a more distal form of stress, childhood trauma, on the DAergic and affective stress reactivity in health and psychosis (chapter 6).

In the realm of daily-life responsiveness to rewards, ESM has offered solid evidence for intact hedonic experience in patients with psychosis (49), a striking finding in the face of the abovementioned deficits in reinforcement learning and deviant DA reactivity to rewards in psychosis. An important consideration of this seemingly contradictory finding is that hedonic capacity is operationalized as the ability to generate *increased*

positive affect in response to pleasurable events, and while a necessary component of successful reinforcement learning, it is not sufficient to ensure it. A more complete picture, however, would be painted if the true essence of reinforcement learning was captured: the ability to *generate behavior* that has previously been associated with increased positive affect. The initial evidence for such propagation of reinforcement learning in the daily life of a large sample of healthy individuals is provided in chapter 4. Finally, daily-life rewards are rarely encountered in vacuum, but rather typically experienced against the backdrop of variable degrees of stress. While experimental evidence points toward noxious effects of stress on reward responsiveness in the laboratory (50, 51), a synergistic approach incorporating real-world examination of these tenets has not been conducted, and will therefore be the subject of chapter 8. The consequences of this research line are far-reaching. In light of the mounting evidence for hyper-reactivity to stress in psychosis, if the stress-associated reward dysfunction does, in fact, translate into the daily life of healthy individuals, it could imply a potentiation of this effect in psychosis.

This dissertation will focus on the neurochemical, behavioral and affective reactivity to stress and reward of healthy individuals, healthy relatives of individuals with psychosis, and patients with psychotic disorder. The first section, named *On reward,* will present findings of dopaminergic reactivity to rewards in individuals with genetic risk for psychosis. Another part of this section will highlight behavioral reactivity to rewards among individuals with high and low negative symptoms of psychosis. In the last part of this section reinforcement learning will be traced to the original affective experience in the daily-life of a large sample of healthy individuals. The second section, *On stress,* will focus first on the effect of acute psychosocial stress on prefrontal dopaminergic activity in patients with psychosis and healthy controls, followed by a chapter on the moderating effect of a distal form of stress, childhood trauma. The next chapter will focus on mapping fear conditioning, an affective correlate of extreme stress response, onto the lesser-investigated parts of the human brain Finally, the last section *On stress and reward* will tie these two lines of work together by providing evidence for the effect of stress on behavioral and affective sensitivity to rewards in healthy individuals, both in the laboratory and in the real-world.

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On reward

Chapter 2

Striatal dopaminergic modulation of reward processing in healthy individuals at a genetic risk for psychosis

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ABSTRACT

Abnormalities in reward learning are a consistent finding in psychotic disorder and have been proposed to be linked to dysregulated subcortical dopaminergic (DA) neurotransmission, which in turn is a suspected mechanism of predisposition to psychosis. Experimental behavioral studies did not, however, corroborate reward dysfunction in individuals at familial risk for psychosis, and functional neuroimaging studies of striatal neural activation to rewards returned nuanced and inconsistent findings in this group. To examine the role of striatal DAergic neuromodulation of reward function in liability to psychosis, we therefore conducted the first functional molecular neuroimaging study of reward processing in the psychosis continuum.

Using a single DA $D_{2/3}$ receptor [^{18}F]fallypride positron emission tomography (PET) scan, we explored the DAergic activity in the striatal (putamen, caudate nucleus [CNC], ventral striatum [VST]) and limbic (hippocampus and amygdala) regions of 16 unaffected first-degree relatives of individuals with psychosis (REL) and 16 gender-, age- and IQ-matched controls during a reward learning task. We detected unaltered reward-induced DA activity in the striatum, hippocampus and amygdala of REL, contradicting the hypothetical prediction of DAergic basis for reward dysfunction in susceptibility to psychosis. Moreover, in relatives and controls alike, greater area of reward-induced DA release in right VST, right putamen and left CNC was associated with better performance on the task. This relationship was not present in hippocampus and amygdala, indicating a specific role of the striatum in the modulation of behavior as a function of outcome, and a preservation of this specificity in individuals genetically predisposed to psychosis. Collectively, these results, along with findings of normative behavioral performance on the reward learning task, as well as absence of (subclinical) positive, negative and depressive symptoms in REL, provide initial hint at adequate reward function in this group.

INTRODUCTION

The capacity to adequately respond to environmental rewards is vital for adaptive functioning, and therefore an essential feature of mental health. Deviations from the norm can give rise to maladaptive approach behavior, and confer susceptibility to psychopathology integral to the psychotic disorder. Positive symptoms, e.g. hallucinations and delusions, are proposed to be occasioned by excessive and aberrant responding to external and internal triggers (1). Negative symptoms, on the other hand, are thought of as the clinical manifestation of impaired reward-seeking behavior: avolition (diminished interest in pursuit of pleasurable experiences), asociality (reduced tendency to participate in social activities) and amotivation (failure to engage or persist in goal-directed behavior) (2). Importantly, this highly debilitating cluster of phenomena transcends the spectrum of psychosis (3), and is shown to precede and predict disease onset in a clinical high risk (4, 5) and even general population (6), suggesting that disturbances in reward-seeking behavior might be a vulnerability trait marker for psychosis.

Impairments in performance on experimental tasks probing reward responsiveness (7-10) and the associated altered striatal modulation of reward processing (11, 12) is one of the most frequently replicated findings in psychotic disorder, spanning across the entire psychosis continuum. Attenuated neural activity in this region during the anticipation of rewards and rewarding feedback has been detected using functional magnetic resonance imaging (fMRI) in an at-risk mental state (13), first-episode (13, 14), medication-naïve (15) and medicated chronic patients (16). The blunted striatal signaling of reward outcome has been receiving special attention in these populations, as it is considered an essential component of prediction error (PE) signaling (10, 14). PEs - outcomes that do not align with predictions (unexpected wins or losses of rewards) - are believed to be registered by striatal dopaminergic (DA) neurons, providing the learning signal that guides future incentive-based choices (17, 18). Blunted PE signaling in the ventral striatum (VST) of patients with psychosis has been thought to undermine value-driven responding, resulting in motivational deficits central to the negative symptoms of psychosis (19).

There is some indication, however, that the unaffected first-degree relatives (REL) of patients with psychosis might represent a departure from this profile of overarching reward dysfunction. Although subtle forms of negative symptoms have been detected in REL (20, 21) (but not always, for example see (22, 23)), this group reports high quality of life (24) and achieves adequate performance on reward learning and conditioning tasks (25, 26). Brain imaging studies echo this somewhat paradoxical pattern by showing that compared to control subjects, REL demonstrate striatal hypoactivity (25, 27) as well as intact activity (26) to reward anticipation, but reward outcomes induce hyperactivity (27) to normal activity (26) in the same region.

In sum, this observational and experimental evidence amasses to two conflicting interpretations: i) the occurrence of attenuated negative symptoms and the associated VST hypo-responsiveness to rewards in REL could be pointing towards a vulnerability trait marker for the motivational impairments integral to psychosis, ii) the healthy status, good adjustment, adequate performance on reward tasks and normal or even supranormal striatal sensitivity to reward outcomes in REL hints at preserved modulation of behavior as a function of feedback, possibly conferring robustness against psychosis in this group. In order to adjudicate between these premises, we explored the neuromodulation of reward processing in unaffected first-degree relatives of patients with psychosis.

Preclinical studies have confirmed the DA neurotransmission as the primary neurochemical component of reward reactivity (28, 29); excitation of the DA neurons in the nucleus accumbens (Nacc), a key portion of the VST, invigorates reward-seeking behavior in rats, while antagonism of their receptors attenuates it (30). This relationship is shown to be reciprocal, as the reward-predictive cues elicit DA release in these neurons, but their excitability is dampened by the blockade of the D_1 or D_2 receptors (31). A landmark study by Pessiglione and colleagues (2006) translated these findings to humans by administering DA medication with opposing effects on DA levels, l-DOPA and haloperidol, to healthy volunteers performing a probabilistic reward learning task designed to generate robust PEs. While the DA-increasing l-DOPA enhanced reward-seeking behavior and the PE signal in the VST, the DA-blocking antipsychotic haloperidol attenuated them (32). Using the same task, a revealing dissociation was also observed in pharmacologically-treated patients with psychosis (33); higher dose of DA-blocking antipsychotic medication was associated with attenuated striatal blood oxygen level-dependent (BOLD) response to PE, whereas intact response was found in patients treated with lower doses (33). These intriguing findings spur the speculation that adequate striatal D_2 blockade normalizes the elevated DAergic tone in psychosis that would otherwise "drown" the phasic bursts to PEs, while leaving enough receptors available to propagate the learning signal (10).

Although the DAergic basis for this mechanism has never been investigated directly in psychosis, a recent meta-analysis of molecular neuroimaging studies confirms functional abnormalities of the subcortical DAergic circuit in this disorder (12). Concretely, increased striatal DA synthesis (34, 35) and release (36, 37) has consistently been detected in psychosis, and replicated in REL (38), implying that the aberrant striatal DA tone in psychosis has, indeed, a genetic component.

In conclusion, there is convincing evidence for the pivotal role of striatal DAergic modulation of normative reward-approach behavior (39, 40), with emerging hints at a dysregulation of this cascade in psychosis. Importantly, despite the evidence for the striatal presynaptic DA abnormality extending to the REL (38), fMRI and behavioral reports suggest that important aspects of the reward function might be spared in this group.

Therefore, in order to explore the putative striatal DAergic modulation of reward learning in relation to genetic predisposition to psychosis *in vivo*, we performed the first functional molecular neuroimaging study of subcortical DAergic activity during reward in the psychosis continuum using positron emission tomography (PET) and the high-affinity and selectivity $D_{2/3}$ ^{18}F-fallypride PET radioligand (41). Specifically, we employed a single ^{18}F-fallypride PET scan (42) during a probabilistic reward task, designed to elicit robust PEs and the associated DAergic activity in the striatal and limbic regions of the brain in a group of healthy first-degree relatives of individuals with a psychotic disorder and healthy controls.

METHODS

Sample and demographics

The RWTH Aachen University ethics committee approved the study. PET approval was additionally granted by the national authority for radiation protection in humans in Germany (Bundesamt für Strahlenschutz, BfS). Written informed consent was obtained before participation after the procedure had been explained, and participants were treated in accordance with the Declaration of Helsinki. Participants were compensated by coupons in the value of 100 euros.

A total of 17 healthy first-degree relatives of individuals with psychosis (REL) and 17 healthy control subjects with no familial history of psychosis were recruited to participate in this study via digital and newspaper advertisements.

The REL group was comprised of individuals who had at least one sibling or parent with a diagnosis of non-affective psychotic disorder given by a psychiatrist, as determined by the Family Interview for genetic Studies (FIGS; NIMH, 1992). The general inclusion criterion for all participants was age between 18-60 years. The general exclusion criteria were i) lifetime history of Axis I or II disorders as determined by the M.I.N.I. (Sheenan, 1990); ii) current use of neuroleptics, steroids, thyroid medication, and lifetime use of illicit hard drugs > 5 times, soft drugs > 20 times, and alcohol > 7 units per week, as confirmed by the M.I.N.I. and by urinalysis on the day of the PET scan; iii) history of any neurological condition, epilepsy or head injury; iv) non-removable metal elements in or on the body; v) vision or hearing impairments affecting the performance on the task; vi) pregnancy, which was confirmed by a urine test on the day of the scan. An additional exclusion criterion specific to the controls was having a first- or second-degree relative with a diagnosed psychotic disorder as determined by the FIGS. Furthermore, the IQ of the sample was ascertained using the Dutch Adult Reading Test (DART), and the level of sub-clinical symptoms of psychosis was measured using the Community Assessment of Psychic Experiences (CAPE) (43).

One relative was excluded based on the use of antidepressants disclosed after inclusion into the study, and one control based on performance on the reward task approaching chance levels, and non-compliance with the study procedures. Therefore, the final analyses were performed on 16 REL (9 women; mean age = 42.38 years, SD = 14.01) and 16 controls (12 women; mean age = 38.06 years, SD = 15.61) matched on gender (X^2 = 1.52, p = .26), age (b = 4.31, t(1,31) = .82, p = .42) and IQ (b = 5.56, t(1,31) = 1.17, p = .25).

Procedures

Upon inclusion into the study and signing of the informed consent, the demographic and lifestyle questionnaires as well as neuropsychological and symptom assessments took place. Thereafter, on the ^{18}F-fallypride PET scan day, all participants underwent first a structural Magnetic Resonance Imaging (MRI) scan, followed by the placement of a catheter into the left antecubital vein. A minimal of 90 minutes were allowed after cannulation for any experience of pain or stress to dissipate, before the participants were positioned on the PET bed, and given a response box with two buttons that would be used to perform the upcoming tasks using the index and middle finger. Afterwards, a transmission scan was performed, followed immediately by the tracer injection. At that moment, the ^{18}F-fallypride PET control condition was initiated, lasting exactly 80 minutes. Then, participants were removed from the PET scanner for a 15-minute break. After repositioning using the localization system of the scanner, a 25-minute baseline rest condition without any stimulation was completed, followed by the experimental probabilistic stimulus selection task (PSST) that was initiated exactly at minute 120 post-injection, and was terminated at the end of the ^{18}F-fallypride PET dynamic acquisition at minute 180. Thereafter, the catheter was removed, and participants were debriefed, compensated and thanked for the completion of the study.

Probabilistic Stimulus Selection Task

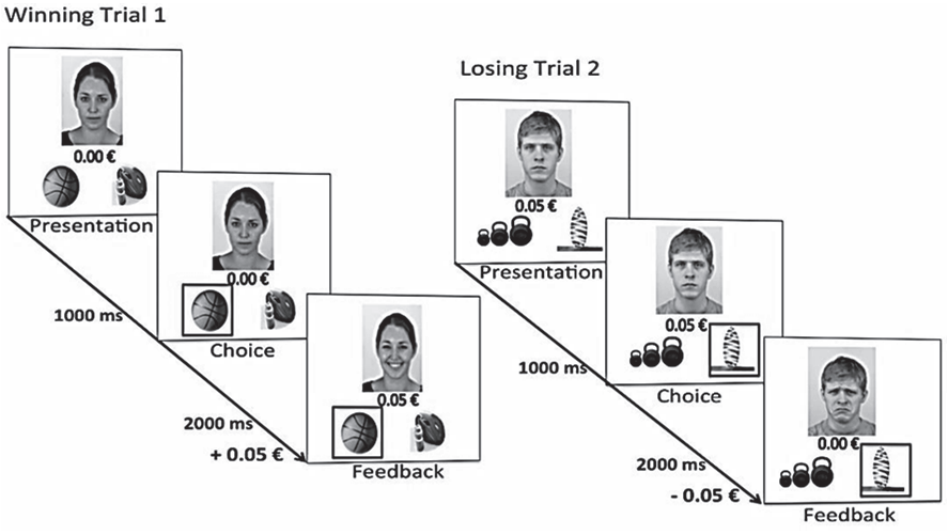
The experimental condition consisted of a version of a probabilistic stimulus selection task (PSST) (32, 44) modified for PET imaging. It was administered using E-prime (Psychology Software Tools), presented on a 30-inch screen. The task was self-paced and consisted of 6 independent learning blocks. In each block 3 pairs of items below a picture of an actor were presented 40 times in a random order, for a total of 120 trials per block. Every trial started with the presentation of a picture of the actor with a neutral expression above a pair of items that illustrated the actor's hobbies (e.g. left item: basketball, right item: bicycle helmet) or profession (e.g. left item: stetoscope for medicine, right item: ruler for mathematics), depending on the block. The same actor was always presented with the same pair of items. A new set of 3 actors + pairs of items was presented in every block, requiring the participants to learn new set of contingen-

cies (figure 1). The images of actors and items were selected randomly from a large pool and were fully counterbalanced across participants. The participants were instructed to learn which picture belonged to each actor by choosing either the left or right item (pressing either the L or R key on the response box) and receiving a feedback: the actor's smile and a win of 5 euro cents following a correct choice, and a frown and the loss of 5 euro cents after the incorrect one. Each pair of items was associated with different probabilities of reinforcement: 90:10, 80:20 and 70:30. For instance, the choice of the correct item of the 80:20 pair led to a smile and +5 euro cents on 80% of the trials and to a frown and a -5 euro cents on 20% of trials. A tally of total money earned was always present in the middle of the screen.

All participants were told beforehand that they would keep the money they earned in the task.

The performance on the PSST was quantified as: 1. the total amount of money each participant won in the task and 2. accuracy in learning the contingencies: average proportion of correct choices (choices of the more frequently rewarded stimulus) on the 90:10, 80:20 and 70:30 pair. To compare the two groups on learning rate throughout the task, all six blocks consisting of 40 trials per pair were divided into four 10-trial sections. Average accuracy per section was then computed, and collapsed across all blocks.

Figure 1: Probabilistic stimulus selection task



Control Task

The control task was designed to contain all features of the PSST, except for the main manipulation, the associative learning from feedback. Similar to the PSST, there were 6

blocks of 120 trials in which the participants were presented with two choice items below a photograph of an actor with a neutral expression. The two choices described some visual feature of the actor, e.g. dark/light hair, oval/long face etc. The participant was required to simply choose one of the items by pressing the L or R key on the response box, and wait for another one to appear, until all 18 actors were presented 40 times, lasting approximately 10 minutes per block. There was a 4 s intertrial interval during which the previous image and items were still visible on the screen. No feedback and therefore no learning occurred in this task, and the participants were explicitly told that there was no right or wrong answer. In total, this task contained the same number of presentations of faces, choices of one of two items, and presses of the response box keys as the PSST, thus controlling for its visuomotor stimulation of the DA system.

Imaging data acquisition and analysis

MRI: T1-weighted MRI scans were acquired on a Siemens 3T scanner (Siemens Healthcare. Munich, Germany) using the Magnetization Prepared Rapid Acquisition Gradient-Echo (MP-RAGE) sequence, with TE = 2.52ms, TR = 1900ms, matrix dimensions = 256 x 256, slice thickness = 1mm, slice number = 176.

Tracer preparation: The radiosynthesis of ^{18}F-fallypride was a high-yield modification of the synthesis method for ^{18}F-desmethoxyfallypride, described in detail elsewhere (45).

PET acquisition: Dynamic ^{18}F-fallypride PET measurements were performed in three-dimensional mode on a Siemens ECAT EXACT HR+ scanner (Siemens-CTY, Knoxville, TN, USA). ^{18}F-fallypride data were collected in a single session (42), starting immediately after a single bolus administration of ^{18}F-fallypride, in 60 s frames during the first 6 min and 120 s frames thereafter. The first segment corresponded to the control and baseline condition and the second segment to the experimental condition (please see above in *Procedures*). Regarding the PET reconstruction, sixty-three slices of 2.4 mm slice thickness (pixel size = 2 mm x 2 mm) were reconstructed per time frame by filtered back projection (Hamm filter) after Fourier rebinning into two-dimensional sinograms. Data sets were corrected for random coincidences, scatter radiation and attenuation (10 min ^{68}Ge/^{68}Ga-transmission scan).

PET data analysis: For each subject, the dynamic PET images were realigned to correct for potential effects of head movement using SPM2 (Wellcome Trust, UK). All the remaining PET processing procedures were performed according to an automatic protocol using the PMOD brain PNEURO tool (v. 3.6, PMOD Technologies Ltd., Zurich, Switzerland). Realigned PET images were first rigidly coregistered to individual T1 MRI. Then the individual MR images were nonlinearly coregistrered to the standard Montreal Neurological Institute (MNI) space MRI template in PMOD. Subsequently the same was done for the PET images using the same spatial transformation as the regis-

tered MR images. For each subject, MR images were segmented into grey matter, white matter and cerebrospinal fluid within native MRI space. Automatic delineation of the left and right cerebellum (reference region, see below) and of the following regions of interest (ROIs): amygdala, hippocampus, caudate nucleus (CNC), putamen and VST, was performed by MRI Parcellation in the PMOD PNEURO tool. All coregistrered and segmented images were visually checked for accuracy. The fit of the delineated regions to the coregistered PET was then visually checked for accuracy, and if necessary, manually adjusted. Subsequently, PET data were analyzed using a modified simplified reference region model (SRRM), the linear extension of the SRTM (LSRRM) (42, 46), in accordance with previous endogenous DA displacement-type experiments (41, 45, 47-49). Reward-induced ^{18}F-fallypride displacement, reflecting DA release (41), was quantified using time-activity curves (TAC) obtained for each ROI and receptor kinetic parameter estimates (42) For each individual, significant ^{18}F-fallypride displacement induced by the reward task was calculated for each ROI as the standardized Z-value of γ (Z = γ/SD[γ]), where γ is considered an additional time-varying parameter in the SRTM kinetic model, estimating the amplitude of ligand displacement during the experimental condition in a single scan session (based on the assumption that changes in competition between DA release and radioligand are reflected in the estimation of γ) (42). The Z-value has been considered an accurate proxy of stimulus-induced changes in DA release (42, 46, 47).

γ was calculated over an exponential decay function $h(t) = \exp[-\tau(t-T)]$, where t = measurement time, T = time of experimental condition initiation (120 min in the current activation paradigm) and τ controls the rate at which activation effects die away (dissipation rate set to $\tau = 0.03$ min^{-1}). The number of voxels surviving p(/number of total voxels) = .05 reflects the spatial extent of significant task-induced ligand displacement (hence DA release) and was used as an additional outcome measure of reward-induced DAergic activity. This approach has been validated for ^{18}F-fallypride and has been used to investigate phasic DAergic activity in extrastriatal and striatal areas (41, 45, 47-50).

Data analyses

All final analyses were performed in STATA 11.2 (StataCorp, 2009). First, to ascertain whether there were group differences in any of the demographic and clinical measures (listed in *procedures)*, a series of regression analyses were performed with group (relatives, controls) as the predictor, and the following separate outcome variables: age, IQ, CAPE subclinical negative symptoms score, positive symptoms score and depressive symptoms score. Gender was the outcome of a logistic regression with group as the predictor.

To compare the two groups on the behavioral performance on the reward task, a regression analysis was performed with total winnings as the outcome variable, and

the group (relatives, controls) as the predictor. Then, three separate regression analyses was performed with the group as the predictor and proportion of correct choices on the 90:10, 80:20 and 70:30 pairs as the outcome variable.

Second, to compare the two groups on the spatial extent of reward-induced DA activity in all ROIs, a series of regression analysis were performed with the percentage of voxels activated per ROI as the dependent, and group as the independent variable. As a support for these analyses, the two groups were also compared on the amplitude of reward-induced DA release in all ROIs, using a series of regression analyses with Z-value of γ of the ROI as the dependent, and group as the independent variable.

To test group differences in the association between reward-induced DA activity in all ROIs and performance on the PSST, regression analyses were conducted with total winnings as the outcome variable, and the amplitude of reward-induced DA release in each ROI, group, and their interaction as the predictors. These analyses were then repeated for accuracy (proportion correct choices) on the 90:10 item, 80:20 item and the 70:30 item as the separate outcome variables. Additionally, to determine the strength and direction of associations between reward-induced DA activity and reward performance separately for relatives and controls, all regressions were repeated for each group separately.

Finally, to investigate group difference in the association between relevant clinical symptoms and reward-induced DA activity in all ROIs, CAPE positive symptoms, negative symptoms, and depressive symptom scores were the predictors, each in a separate regression analysis, along with group and the interaction between the symptom score and group, with the spatial extent / amplitude of reward-induced tracer displacement as the outcome. The same regressions were then performed for each group separately. To control for age, gender and IQ, these variables were entered into the regression as additional predictors in all the analyses.

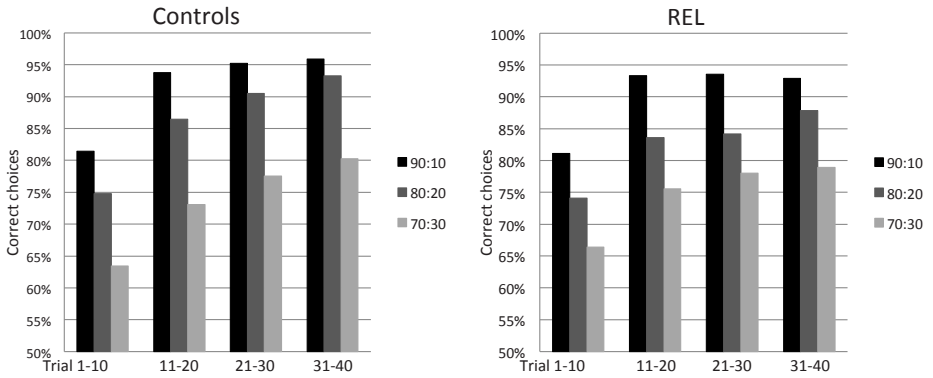
RESULTS

Demographics and behavioral performance on the Reward Task

As specified in the *Sample and demographics* section, there were no differences between the two groups on any of the demographic variables, nor in subclinical positive symptoms of psychosis (b = .053, t(1,31) = .71, p = .367), negative symptoms of psychosis (b = .121, t(1,31) = .92, p = .485) and depressive symptoms (b = .195, t(1,31) = 1.2, p =. 241). The two groups demonstrated satisfactory and comparable performance on the reward task. There was no group difference between total winnings (b = .011, t(1,31) = .11, p = .912), ranging from 6.75 to 17.2 euros earned, with controls and REL earning on average 12.46 euros (SD = 2.89) and 12.57 euros (SD = 2.65), respectively. The two groups did not differ in accuracy on the 90:10 item (b = -.004, t(1,31) = -.14, p

= .892) nor on the 80:20 item (b = .018, $t(1,31)$ = -.42, p = .677), but there was a trend for worse performance of REL on the 80:20 item (b = -.053, $t(1,31)$ = -1.91, p = .066; Graph 1)

Graph 1: Accuracy on all three pairs throughout the task per group



All six blocks each consisting of 40 trials per pair of items (90:10, 80:20, 70:30) were divided into four sections of 10 trials. Accuracy was computed as the average proportion of correct choices in each 10-trial section of all blocks combined. Both controls and REL demonstrated comparable, increasing accuracy (here presented as percentage correct choices) on all three pairs during the task. Both groups exceeded chance level performance within the first 10 trials of the blocks.

Reward-induced increase in Striatal Dopamine Activity During Reward Task

In the healthy controls, significant reward-induced increase in radioligand displacement and the spatial extent of radioligand displacement was detected in striatal (CNC, putamen) limbic striatal and limbic (hippocampus and amygdala) ROIs (table 1). In the REL, significant reward-related increase in ligand displacement, as well as its spatial extent was observed in all ROIs, except for left CNC and right putamen (table 1).

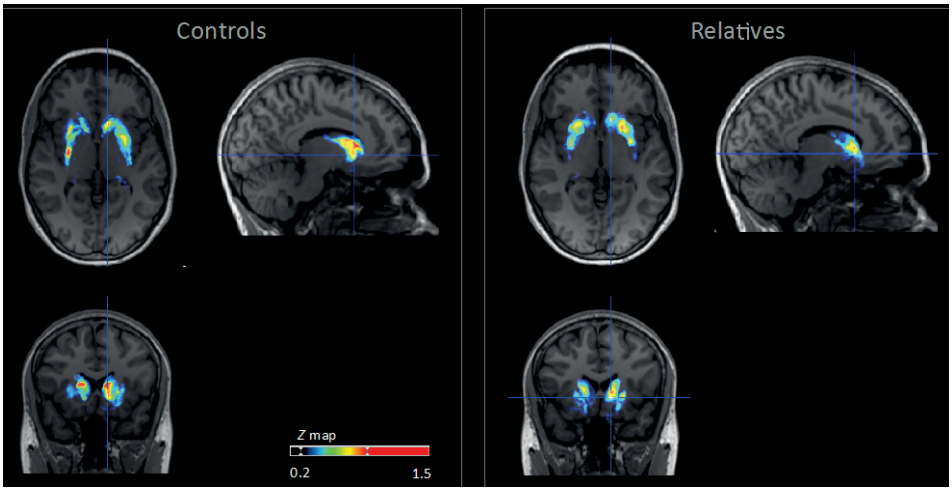
Importantly, there was no difference between groups in the intensity of reward-related tracer displacement nor in its spatial extent in any of the ROIs (all p > .05; table 1; figure 2), suggesting a comparable DAergic activity during reward processing for both groups in all significantly involved striatal ROIs.

Table 1: Spatial extent and amplitude of reward-induced tracer displacement in controls and REL per ROI

	Controls (*N*=16)		REL *(N*=16)		Group Difference		
ROI	M	SD	M	SD	p-value	β	t
Spatial extent of reward-induced tracer displacement (% voxels)							
R Hippocampus	34.302	24.403	33.178	26.61	0.715	-3.59	-0.37
L Hippocampus	35.222	23.063	33.927	22.428	0.62	-4.32	-0.5
R Amygdala	24.966	22.759	35.96	28.519	0.508	7.73	0.67
L Amygdala	24.19	18.901	36.991	38.239	0.402	9.11	0.85
R CNC	17.168	16.883	17.573	19.71	0.741	-2.27	-0.33
L CNC	20.586	20.881	12.828	14.818	0.169	-9.66	-1.41
R Putamen	19.323	21.715	15.68	22.031	0.395	-6.96	-0.86
L Putamen	16.59	19.229	17.685	20.763	0.957	-0.41	-0.05
R VST	23.786	27.807	17.069	17.033	0.356	-8.01	-0.94
L VST	17.022	16.096	11.403	16.846	0.147	-8.86	-1.49
Reward-induced tracer displacement (Z-value of gamma)							
R Hippocampus	0.00714	0.02804	0.01088	0.02118	0.907	0.001	0.12
L Hippocampus	0.00981	0.02294	0.01325	0.02047	0.817	0.002	0.23
R Amygdala	0.00267	0.01855	0.01349	0.02091	0.178	0.011	1.38
L Amygdala	0.00222	0.02222	0.01204	0.02054	0.305	0.008	1.05
R CNC	0.0059	0.00785	0.00446	0.02196	0.249	-0.006	-1.18
L CNC	0.00244	0.01279	-0.00022	0.01727	0.38	-0.005	-0.89
R Putamen	0.00623	0.01947	-0.00308	0.01812	0.69	-0.013	-1.9
L Putamen	0.00041	0.01119	0.00256	0.01815	0.911	-0.001	0.11
R VST	0.00691	0.01227	0.00909	0.0196	0.997	-0.00002	0
L VST	0.00573	0.01448	0.00376	0.01026	0.335	-0.005	-0.98

Spatial extent and amplitude of reward-induced ^{18}F-fallypride displacement (in the upper and lower part of the table respectively) in limbic (Hippocampus and Amygdala) and striatal regions (CNC, Putamen, VST) per group (controls and REL), and differences between groups. REL = healthy first-degree relatives of patients with psychosis; M = mean; SD = standard deviation; *B* = beta coefficient; *t*=t-statistic; CNC = caudate nucleus; VST = ventral striatum; R = right; L = left.

Figure 2: Striatal reward-induced dopaminergic activity in the VST of controls and REL



Average statistical parametric Z-map per group (controls and relatives) of γ representing the striatal dopaminergic activity induced by the reward learning task shown in transverse, coronal, and sagittal sections overlaid on T1-weighted MRI template. The images visualize the comparable striatal reward-induced ^{18}F-fallypride displacement in controls and relatives, REL.

Correlation Between Reward-Induced Dopamine Activity and Reward Task Performance

There was no significant group x spatial extent of reward-induced increase in radioligand displacement on reward performance (total winnings) in any of the ROIs (all p > .05; all p > .05 also for intensity of reward-induced tracer displacement).

To the contrary, both groups demonstrated positive association between DA activity and total winnings. In REL, statistical significance was reached in right VST (b = .08, t(15) = 2.51, p = .029), left putamen (b = .075, t(15) = 2.32, p = .033), and a trend-level association emerging in right CNC (b = .062, t(15) = 1.98, p = .074). In controls, statistically significant positive association between DA activity and performance on the reward task was present in left putamen (b=.087, t(15)=2.33, p=.037), and right CNC (b=.094, t(15)=2.33, p=.040), with a trend for significance observed in right VST (b=.054, t(15)=1.86, p=.090).

Importantly, there were no group x DA activity interactions on reward performance in bilateral hippocampus (right: b=.027, t(1,31)=.66, p=.518; left: b=-.003, t(1,31)=-.07, p=.942) and amygdala (right: b=.007, t(1,31)=.22, p=.830; left: b=.027, t(1,31)=.62, p=.541), nor were there significant or trend-level associations between task performance and task-induced DA activity in REL or controls in these regions (all p > .05).

Similarly, there was no significant group x spatial extent of reward-induced increase on tracer displacement on accuracy (proportion correct choices) on either of the pairs

(all $p > .05$). Both groups demonstrated positive associations between accuracy on the three pairs and reward-induced tracer displacement in R CNC, L Putamen, bilateral VST, L Hippocampus and bilateral amygdala, but only associations in striatal ROIs reached significance or trend level (table 2, figure 3). A trend-level negative association between accuracy on the 70:30 pair and reward-induced tracer displacement emerged in the R Hippocampus for both controls and REL (table 2).

Table 2: Associations between reward-induced 18^{F}-fallypride displacement in all ROIs and accuracy on all three pairs of the reward task (proportion correct choices)

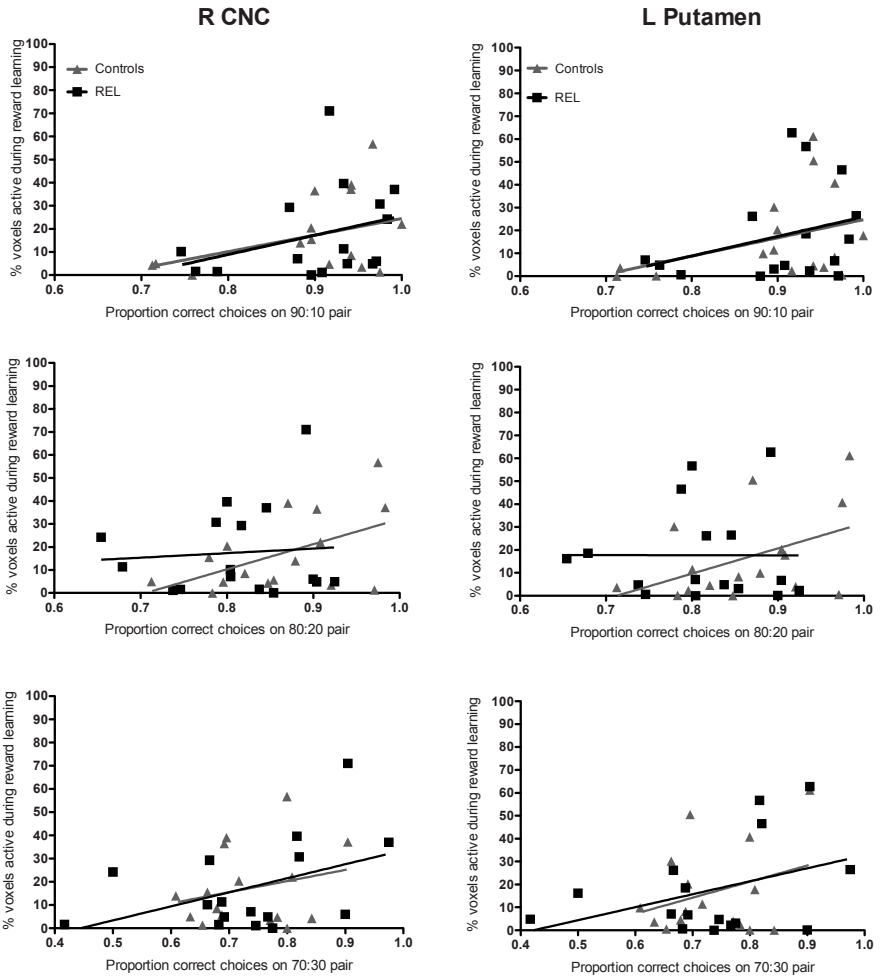
	CONTROLS									RELATIVES								
Pair	90 : 10			80 : 20			70 : 30			90 : 10			80 : 20			70 : 30		
ROI	*p-value*	*B*	*t*	*p-value*	*B*	*t*	*p-value*	*B*	*t*	*p-value*	*B*	*t*	*p-value*	*B*	*t*	*p-value*	*B*	*t*
R CNC	0.036**	0.0029	2.380	0.008**	0.0030	3.240	0.451	0.0011	0.780	0.115	0.0019	1.710	0.125	0.0017	1.660	0.079*	0.0038	1.940
L Putamen	0.029**	0.0028	2.510	0.014**	0.0026	2.910	0.389	0.0012	0.900	0.069*	0.0019	2.020	0.356	0.0009	0.960	0.057*	0.0037	2.130
R VST	0.006**	0.0024	3.440	0.288	0.0010	1.120	0.757	0.0003	0.320	0.082*	0.0022	1.92	0.189	0.0016	1.4	0.063*	0.0044	2.070
L VST	0.087*	0.0026	1.880	0.059*	0.0025	2.1	0.407	0.0013	0.86	0.666	0.0006	0.440	0.494	0.0009	0.710	0.798	0.0007	0.260
R Hpc	0.980	0.0000	-0.030	0.612	-0.0005	-0.520	0.056*	-0.0020	-2.130	0.134	0.0013	1.62	0.552	0.0005	0.610	0.077*	-0.0030	-1.950
L Hpc	0.371	0.0010	0.930	0.247	0.0012	1.220	0.761	-0.0004	-0.310	0.288	0.001	1.12	0.279	0.0009	1.140	0.139	0.0025	1.590
R Amygdala	0.597	0.0006	0.540	0.806	0.0003	0.250	0.219	-0.0014	-1.300	0.255	0.0007	1.2	0.890	-0.0001	-0.140	0.300	0.0012	1.090
L Amygdala	0.997	0.0000	0.000	0.722	0.0006	0.370	0.396	-0.0015	-0.880	0.376	0.0006	0.92	0.642	0.0003	0.480	0.154	0.0018	1.530

R = right, L = left; CNC = caudate nucleus, Hpc = Hippocampus; *B* = beta coefficient; *t*=*t*-statistic

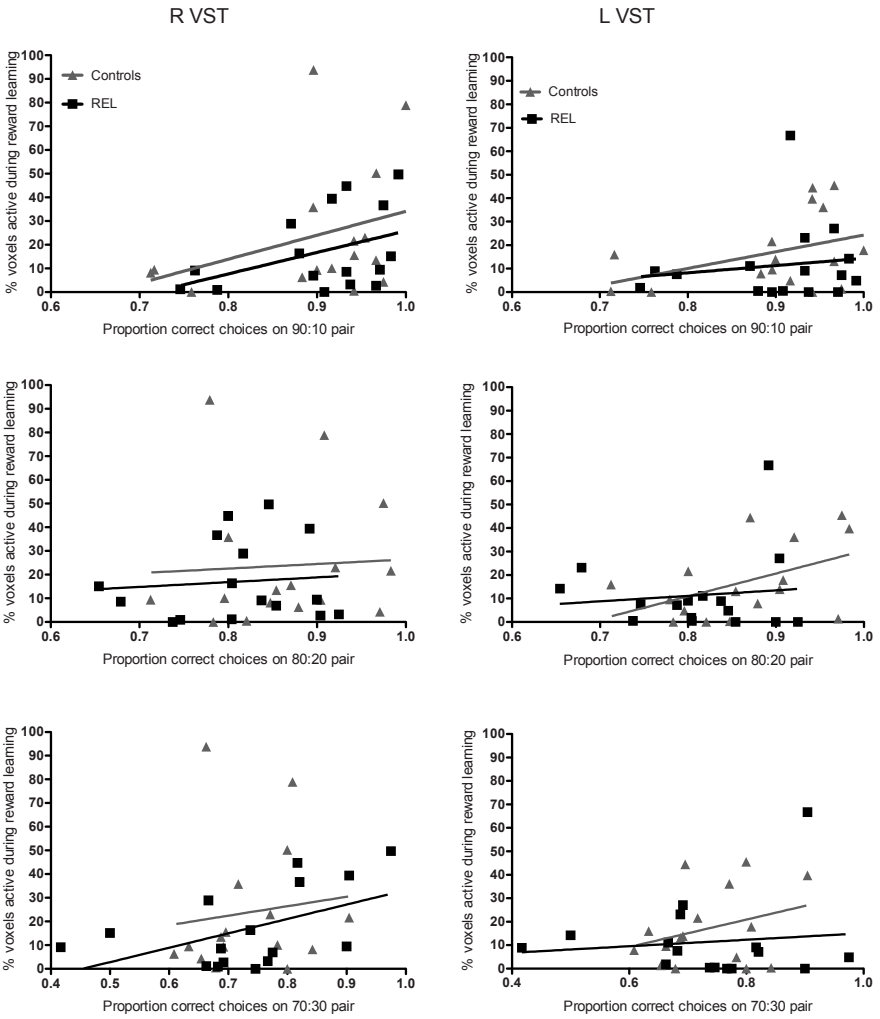
** significance at p<0.05

*trend for significance

Figure 3: Associations between reward-induced 18^{F}-fallypride displacement in the striatal ROIs and accuracy on all three pairs of the reward task (proportion correct choices)



R = right; L =left, CNC = caudate nucleus



R = right; L =left, VST = ventral striatum

Correlation Between Reward-Induced Dopamine Activity and Subclinical Measures of Psychosis

The regression analyses did not reveal any group x spatial extent of reward-induced increase in tracer displacement interaction on CAPE subclinical negative, positive and depressive symptom score, in any of the ROIs (all $p > .05$), nor was there a group x intensity of reward-induced tracer displacement interaction on any of the clinical measures (all $p > .05$). Likewise, none of these associations were present in any ROI of REL or controls separately (all $p > .05$), indicating a lack of a connection between reward-related striatal and limbic striatal DA activity and subclinical symptoms of psychosis in unaffected individuals.

DISCUSSION

The present study provides the first functional molecular neuroimaging account of DAergic activity during reward learning in the psychosis continuum – individuals with familial risk for psychosis, and the first exploration of this mechanism in healthy controls using a highly selective and specific $D_{2/3}$ radiotracer ^{18}F-fallypride. Specifically, we investigated the DAergic activity during prediction error-based responding, an essential requisite of motivated action (51), and detected unaltered PE-induced DA activity in the striatum, hippocampus and amygdala of healthy first-degree relatives of patients with psychosis (REL). In regards with these findings, however, it is important to note that the REL differed qualitatively from controls in that as a group, they showed no significant increase of reward-induced DA activity in the left CNC and R putamen, and numerically, they showed smaller spatial extent of reward-induced DA activity in the striatum. Neither of these differences approached a trend level of statistical significance, even though the sample was associated with a power to detect group differences of 0.87, and was more substantial than samples typically reported in comparable studies (48, 50, 52).

Importantly, in REL and controls alike, greater area of reward-induced DA release in right VST, right putamen and left CNC was associated with better performance on the task. This relationship was not present in hippocampus and amygdala, indicating a specific role of the striatum in the modulation of behavior as a function of outcome, and a preservation of this specificity in individuals genetically predisposed to psychosis. This interpretation is further supported by findings of normative behavioral performance in the reward learning task, as well as the absence of (subclinical) positive, negative and depressive symptoms in the REL group.

The findings of adequate reward learning add to the growing evidence for intact capacity to acquire reward contingencies in REL (25, 26). Furthermore, the finding of specific and appropriate modulation of this process by striatal DA aligns with all existing, albeit sparse, fMRI studies of reward processing in this group reporting normal (26) or supranormal (27) striatal BOLD signal to reward feedback. Reward anticipation, on the other hand, has been shown to elicit hypoactivation (25, 27), but also normal activation (26) in this group. This dissociation is especially noteworthy considering that in the reward learning task employed in the current experiment, performance depends largely on the ability to learn from reward feedback, specifically in errors in predictions thereof, with minimal contribution of sensitivity to reward cues (44). Complementary insight can also be derived from pharmacological manipulation study using DA-enhancing versus DA-blocking agents in healthy individuals performing a similar task (32). In a complete agreement with our findings, pharmacologically-increased DA levels were associated with better performance on the task and greater ventral striatal activation to PEs (32), corroborating the essential role of striatal DAergic modulation of reward learning detected in both of our groups. Meanwhile, the group receiving DA

antagonists demonstrated attenuated VST activity to PEs and poorer performance (32), echoing results from chronic unmedicated psychosis patients (14, 15). Relatively sparse DA blockade, however, has been shown to normalize both deficits in patients (33, 53). These reports suggest that instead of a striatal hypodopaminergia, as one might infer from the pharmacological manipulation study, in psychosis, abnormally *increased* DA tone in the striatum could likely "drown" the phasic bursts to PEs, preventing the reward teaching signal to be registered and inform future actions (19).

Our results thus offer initial hint at neurochemical divergence from this proposed mechanism in familial risk for psychosis. However, in view of the aforementioned numerically smaller magnitude and extent of reward-induced DA activity in REL compared to controls, replication studies are vital to decisively substantiate this phenomenon. Additionally, the current results should be interpreted with due consideration of other limitations of the study. Firstly, the assumptions of the model used to analyze the PET imaging data constrain the order of the conditions to the control condition always being followed by the experimental condition. This design was held constant for all participants, and might have affected the results if the REL were more sensitive to fatigue or other burden posed by the PET scan compared to controls. This is not probable, however, considering that the REL group was, identically to the controls, free of psychopathology, cognitive impairment or performance deficits. Additionally, this issue would be of a greater concern if there was an indication for a group difference in the intensity or spatial extent of DAergic activity in response to rewards, but this was not the case, and the task order had, therefore, likely equal effect on both groups. Secondly, to ensure that the experimental reward task induced the most robust DAergic activity possible, all participants were complete novices to probabilistic reward learning at the outset of this condition, without any prior training on the task. All participants had thus only a few minutes immediately before the experimental condition to read the instructions and ask questions to the experimenter, which might have created unequal conditions for REL group if their ability to grasp instructions was inferior to that of the controls. This is also unlikely, however, because the REL group not only demonstrated optimal learning of the reward contingencies, but numerically slightly outperformed the controls. Moreover, the REL group had, on average, numerically higher estimated IQ than the controls, thus minimizing the chance that this group was at a cognitive or intellectual disadvantage.

Finally, the other side of these arguments for the REL group being comprised of high-functioning adults without any psychopathology is that they might not be fully representative of the entire group of the first-degree relatives of patients with psychosis, particularly when compared to the large-scale study of Grimm and colleagues (2014) with more than 50 individuals that did report group differences in VST BOLD activation to rewards (25). Although we attempted to minimize any selection bias by recruiting all participants exclusively via newspaper and online advertisements, these individuals were self-selected to undergo a demanding experiment, and therefore

possibly on the higher end of the spectrum of mental resilience and clinical health, as also confirmed during the rigorous screening procedure. Nevertheless, this also holds true for the controls, giving us solid reasons to believe that the group composition and comparison was justified and the findings merited.

In conclusion, we provide initial evidence for unaltered and specific striatal DAergic activity during reward processing in healthy first-degree relatives of individuals with psychosis. Complementary neuromolecular studies probing the subcortical DAergic contribution to reward learning in individuals further on the psychosis continuum are warranted to elucidate the precise nature of the putative deviation from optimal modulation of behavior as a function of reward at the synapse, in order to facilitate the design of rational strategies to intervene at this level.

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Chapter 3

Optimizing vs. Matching: Response Strategy in a Probabilistic Learning Task is associated with Negative Symptoms of Schizophrenia

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ABSTRACT

Previous research indicates that behavioral performance in simple probability learning tasks can be organized into response strategy classifications that are thought to predict important personal characteristics and individual differences. Typically, relatively small proportion of subjects can be identified as optimizers for effectively exploiting the environment and choosing the more rewarding stimulus nearly all of the time. In contrast, the vast majority of subjects behaves sub-optimally and adopts the matching or super-matching strategy, apportioning their responses in a way that matches or slightly exceeds the probabilities of reinforcement. In the present study, we administered a two-choice probability learning paradigm to 51 individuals with schizophrenia (SZ) and 29 healthy controls (NC) to examine whether there are differences in the proportion of subjects falling into these response strategy classifications, and to determine whether task performance is differentially associated with symptom severity and neuropsychological functioning. Although the sample of SZ patients did not differ from NC in overall rate of learning or end performance, significant clinical differences emerged when patients were divided into optimizing, super-matching and matching subgroups based upon task performance. Patients classified as optimizers, who adopted the most advantageous learning strategy, exhibited higher levels of positive and negative symptoms than their matching and super-matching counterparts. Importantly, when both positive and negative symptoms were considered together, only negative symptom severity was a significant predictor of whether a subject would behave optimally, with each one standard deviation increase in negative symptoms increasing the odds of a patient being an optimizer by as much as 80%. These data provide a rare example of a greater clinical impairment being associated with better behavioral performance.

INTRODUCTION

Patients with schizophrenia demonstrate a range of cognitive, motivational and affective deficits that limit their adaptive functioning. In the recent literature there has been a renewed focus on the role of basic reward processing mechanisms that could theoretically be related to both cognitive and motivational impairments. Of particular interest is the finding that abnormal reward processing is associated with greater severity of both positive and negative symptoms. For example, Corlett and colleagues (1, 2) and Murray and others (3) have found that abnormal processing of positive feedback may be related to the severity of positive symptoms, a finding that fits with the predictions that emerge from Kapur's notion that abnormal dopamine release would lead to context-inappropriate attributions of salience (4). In contrast, we and others found that abnormalities in reinforcement learning and decision-making (5-8), and associated neural signals (9) appear to be linked to negative symptoms.

One problem of the available behavioral evidence is that most of the experiments have involved somewhat complex tasks, and it is possible that non-reward-related cognitive impairments may have affected performance. To address that limitation, we used a very simple two-choice probability learning task in which one choice was rewarded 70% of the time, and the alternative was reinforced only 30% of the time. One interesting feature of such simple tasks is that they tend to elicit non-optimal decision making, in that people often allocate their response choices to match the probability levels of the more frequently rewarded stimulus. That is, rather than choosing the stimulus that on any given trial has the highest expected value in order to maximize overall payoff, people often allocate approximately 70% of their responses to this stimulus, a phenomenon described first by Herrnstein and termed "matching" (10-12). It is noteworthy that there is evidence that many non-human animals (rats, birds, monkeys) reliably demonstrate this behavior, suggesting that higher order cognitive processes are not an essential factor contributing to the widely observed and arguably universal sub-optimal performance (12, 13).

This issue of response strategy has been extensively studied in humans, with evidence indicating that healthy individuals most frequently rely on a matching strategy (14-18), and to a lesser degree on a super-matching strategy (19-22), in which rates of choosing the optimal response overshoot the reinforcement rate of that response. Although these are the most common response strategies employed in two-choice probability learning tasks, behavior ranging from chance (50% allocation to each alternative) to maximization (100% allocation to the rewarding alternative) has also been observed (23-25). To explore factors associated with the formulation of these different response strategies, Shanks and colleagues (25) conducted a series of probabilistic learning experiments in which they manipulated variables such as number of trials, frequency and nature of the feedback and monetary payoff. Their results suggest that about 75% of subjects can achieve maximization if provided with monetary incentive

and other meaningful feedback about their performance, and the task has a large enough number of learning trials. The authors hypothesized that the behavior of the remaining 25% of subjects (i.e., those who did not reach a level characteristic of maximization and were thus immune to these task manipulations) could be explained by internal factors and individual differences such as sensitivity to feedback, cognitive functioning, proneness to boredom, risk-aversion, and utility representation. However, factors underlying suboptimal performance on these two-choice probability learning tasks remain unresolved, as they have yet to be systematically examined in an empirical study.

Contributing factors affecting subjects' performance can be expected to be population-specific, and in the case of individuals with schizophrenia consist of any number of the core illness dimensions associated with the disease (e.g., positive symptoms, negative symptoms and cognitive impairment (26)). Our observations that patients with more severe negative symptoms show impairments in learning from positive feedback (5, 8) might lead one to predict that such patients would perform sub-optimally on a two-choice probability learning task. On the other hand, computational modeling evidence from our group (27) suggests an association between greater negative symptom severity and reduction in meaningful exploration of the environment that leads to perseveration. Thus, in the context of the two-choice task environment, one might be tempted to predict that higher levels of negative symptoms would be related to paradoxically superior performance. That is, a reduction in exploration would lead the high negative symptom patients to stick with a winning response, resulting in higher overall earnings. It is difficult, a priori, to adjudicate between these two predictions. Thus, we designed a simple probability learning task to directly test these competing hypotheses.

METHODS

Participants

Fifty-one patients meeting DSM-IV criteria for schizophrenia or schizoaffective disorder (SZ), and twenty-nine healthy control (NC) subjects volunteered to participate in this study, which was approved by the University of Maryland School of Medicine Institutional Review Board. All participants provided informed consent and received monetary compensation for their participation in the study.

Individuals with SZ were clinically and medically stable outpatients of the Maryland Psychiatric Research Center, as determined by their psychiatrist, therapist and clinical documentation. All patients were receiving antipsychotic medication and were on a stable regimen for a minimum of four weeks prior to entering the study. Almost all

patients were being treated with second-generation antipsychotics (see Table 1 for subject demographic, clinical, and neuropsychological assessment data).

All SZ patients were rated for clinical symptoms based on interviews conducted by trained case-workers, using the following measures: the Brief Psychiatric Rating Scale (BPRS) (28), the Scale for the Assessment of Negative Symptoms (SANS) (29), and the Calgary Depression Scale (CDS) (30). Negative symptom ratings from the SANS were used to divide patients into a high negative (HN) symptom group and a low negative (LN) symptom group. In order to do this, we determined the median SANS total score for the entire patient sample (28). All patients with a SANS total score lower than 28 were assigned to the LN group (N=25) and all patients with a SANS total score greater than, or equal to, 28 were assigned to the HN group (N=26; subjects whose SANS total scores fell at the median were added to the smaller group).

Normal control (NC) participants were recruited from the community via random digit dialing and word of mouth (from those recruited by random digit dialing). All NC participants had no current Axis I or II diagnoses, as determined by the Structured Clinical Interview for DSM-IV (SCID), no family history of psychosis, and were not taking any psychotropic medications. In addition, all study participants denied substance abuse within the past 6 months and had no lifetime history of neurological disorder.

Patients and controls were matched on age [$t(68)=0.246$], parental education [$t(61)=1.132$], ethnicity [$\chi^2(4)=0.855$], and gender [$\chi^2(1)=0.797$]. All participants completed a standard battery of neuropsychological tests, symptom interviews, and computerized reward learning tasks (including the two-choice probability learning task). Neuropsychological tests included the MATRICS battery (31) Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999), Wide Range Achievement Test (WRAT; Wilkinson, 1993), and Wechsler Test of Adult Reading (WTAR; Wechsler, 2001). Patients and controls did not differ significantly in WRAT [$t(67)=1.779$] and WTAR [$t(68)=1.878$]. However, patients had lower WASI estimated IQs [$t(68)=5.053$] and MATRICS Overall scores [$t(65)=4.602$].

Table 1: Demographic information and cognitive assessments for patients (N=51) and controls (N=29)

Measure	Control M (SD)	LN Patient M (SD)	HN Patient M (SD)	p-value
Age	44.81 (10.49)	43.79 (8.67)	43.88 (10.64	0.917
Education (years)	15.03 (2.23)	13.08 (2.14)	12.56 (2.45)	<0.001
Paternal Education (years)	12.82 (3.43)	14.46 (3.67)	13.16 (3.72)	0.245
Gender (M: F)	21:8	17:8	19:6	0.853
Race				0.579
African American	10	8	9	
Caucasian	19	12	16	
Other	0	5	0	
Standard Neuropsychology				
WRAT	101.03 (17.25)	94.44 (15.11)	90.52 (12.51)	0.041
WTAR	103.52 (17.15)	96.56 (17.29)	93.36 (17.36)	0.091
WASI	113.52 (13.40)	97.04 (15.45)	95.24 (12.15)	<0.001
MATRICS battery	48.33 (15.24)	31.80 (14.12)	29.32 (13.17)	<0.001
Antipsychotic Medication Regimen				
Haloperidol or Fluphenazine only	-	0	1	
Clozapine only	-	6	9	
Other second-generation only	-	8	11	
Clozapine + another antipsychotic	-	5	3	
First -generation + second generation antipsychotic	-	0	1	
Clinical Ratings				
BPRS total score	-	34.72 (7.15)	37.56 (9.26)	0.231
SANS total score	-	19.16 (8.72)	35.60 (9.06)	<0.001
Calgary Depression Scale	-	1.84 (1.97)	2.56 (2.71)	0.288

Two-Choice Probability Learning Task

Participants performed a simple probability learning task in which they were presented with a pair of identical items centered vertically on either side of a computer screen. The stimuli were treasure chest boxes presented on a black-colored background. The participants were asked to press a button on a response pad to select one of the two treasure chests. The left button corresponded to the left treasure box and the right button to the right treasure box. The selection of the treasure chest on one side was reinforced on 70% of trials and the selection of the treasure chest on the other side was reinforced on 30% of the trials. The location of the more frequently reinforced treasure box was counterbalanced across all participants and once assigned, it remained constant throughout the task. If the participant selected the winning item, the chosen treasure box was replaced by a nickel, coupled with the word “win” and a cash register sound. If the selected item did not win, the treasure chest remained on the

screen and the words “Not a winner, better luck next time” were displayed (without any accompanying sound). The participants were not informed of the actual probabilities of reinforcement, and the instructions indicated that there was no cue, pattern or system that could be used to earn a coin on each trial. However, in order to help the participants decide on the best strategy, they were advised to sample both of their options sufficiently, pay attention to the outcome of their choices, and learn from experience. All participants completed a brief practice session consisting of 5 trials to ensure that they understood the instructions and had an opportunity to ask questions. Subsequently, a total of 300 trials were administered in one session, divided into 6 blocks of 50 trials. All trials were response terminated and the task took approximately twenty minutes to complete, with short breaks between blocks. Participants were able to view their running tally of money earned during the task via a display box located in the left corner of the computer screen.

Data Analysis

The classification of response strategies was based upon previous research using similar two-choice reinforcement learning tasks (25, 32, 33) that have generally classified performance according to 4 categories thought to reflect different strategies: 1) random chance, possibly reflecting failure to learn; 2) matching the reward probability 3) super-matching, overshooting the reward frequency of the best choice or, 4) optimizing, a strategy of almost always selecting the best response.

We used a five-stage iterative procedure to assign subjects to performance classes. Because we did not know the true probabilities of choosing the best response corresponding to the super-matcher and optimizer response strategies, we made initial classifications of participants using binomial expansion of our initial estimates of the probabilities corresponding to each classification, followed by maximum likelihood estimation to assign subjects to categories based on performance during the middle 100 trials. We then determined the mean probability of optimal choice associated with each performance class and performed binomial expansions of those probabilities, before re-classifying subjects based on maximum likelihood estimation (see Supplementary Materials for details on the classification procedure).

Since the four performance groups can be ranked in order of approach to the optimal strategy (chance< matchers<supermatchers<optimizers), we compared the two groups on degree of optimality in their strategy using a Mantel-Haenszel chi-square test (34) difference in average rank order of strategies. After classifying subjects into the four performance groups, we excluded the subjects who performed at chance levels from further analyses [n=2/29 controls, 8/51 patients]. Subsequently, we employed a two-way analysis of variance test (ANOVA) to determine whether there were differences in proportions of patients and controls in each class. Additionally, we proceeded to perform a series of t-tests and one-way ANOVAs on neuropsychological and

clinical measures using performance classification as a between-subjects factor. We then analyzed correlations between experimental performance measures and clinical and neuropsychological functioning of both patient and control groups. Subsequently, we standardized the BPRS and SANS scales by computing z-scores in order to perform binomial regression analyses, which allowed us to examine the extent to which positive and negative symptoms predict behavioral task performance.

RESULTS

Comparison of Overall Learning between Patients and Controls

There was no significant difference between normal controls and patients in the proportion of trials in which they chose the optimal side (Figure 1 and 2), with no significant difference in overall performance [control mean=0.78, SD=0.10; patient mean=0.74, SD=0.10; $t(68)=1.595$, $p=0.115$], early learning [first hundred trials; NCs: 0.69 ± 0.13; SZs: 0.65 ± 0.10; $t(68)=1.546$, $p=0.127$], or end performance [last hundred trials; NCs: 0.83 ± 0.11; SZs: 0.80 ± 0.12; $t(68)=1.254$, $p=0.214$]. Patients and controls also showed similar increases in their proportions of choices of the optimal side from the first to the last hundred trials [t(68)=0.195].

Figure 1: Proportion of SZ patients and controls in each performance group

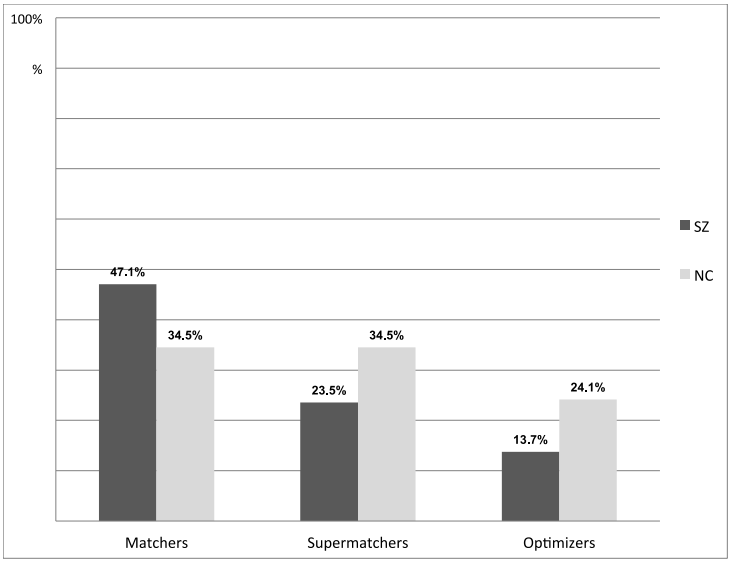
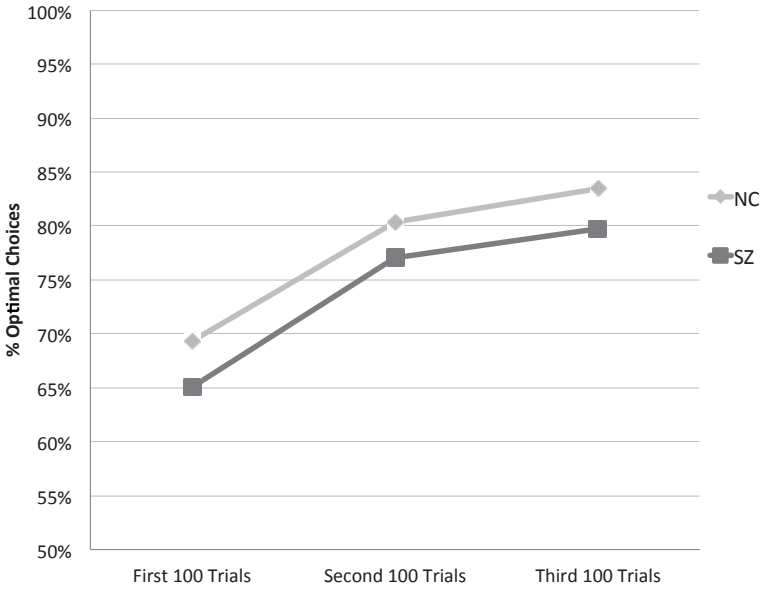


Figure 2: Average percentage of optimal choices of SZ patients and controls in all three blocks.



Proportion of Subjects meeting Criteria for each Response Style

We then classified participants into the four response style categories (random chance, matchers, super-matchers, optimizers), and examined differences in the proportion of SZ and NC participants who fell into these classifications. Overall, the NC group showed a trend toward a greater proportion of subjects meeting criteria for the better response strategy classifications (supermatchers, optimizers) than patients [Mantel-Haenszel $\chi^2(1)= 3.53$, p=0.06].

Comparison of SZ and NC Response Style Groups on Neuropsychological Functioning and Symptom Severity

As noted in the introduction, one can be led to make predictions about learning performance as a function of symptom status. To address this issue, we worked "backwards" by using end performance (last hundred trials) to form distinct groupings, and examined the symptom differences in these classes. As seen in Table 2, the optimizing group of patients had higher levels of negative symptoms. A one-way ANOVA indicated that the matchers, super-matchers, and optimizers significantly differed in the severity of SANS total negative symptoms [$F(2,42)=7.30$, $p=0.002$]. Follow-up, LSD post hoc comparisons indicated that patients within the optimizing group ($M=39.14$, $SD=15.41$) had a significantly greater severity of negative symptoms than those classified as matchers ($M=27.87$, $SD=8.49$) and super-matchers ($M=19.67$, $SD=11.46$). Thus,

more severe negative symptoms were associated with greater optimizing, which results in greater overall earnings in the task, a rare instance of better performance being associated with greater symptom severity. Additional analyses revealed that, when classified according to performance, patients differed in the severity of some negative symptoms, but not others. Specifically, differences between performance subgroups were observe for Affective Blunting [F(2,39)=4.54, p=0.02], Avolition [F(2,39)=3.38, p=0.04], and Anhedonia/Asociality [F(2,39)=4.99, p=0.01], but not Alogia [F(2,39)=1.93, p>0.10].

However, as seen in Table 2, a parallel result was observed with positive symptoms. The analysis of variance revealed significant differences in BPRS total score, [*F*(2,42)=5.998, *p*=0.005], as well as the positive cluster [*F*(2,42)=4.352, *p*=0.020] and negative cluster [*F*(2,42)=3.381, *p*=0.044] scores. No group differences were observed for disorganization cluster scores [*F*(2,42)=1.69, *p*=0.198]. Positive symptom scores in optimizers (M=3.04, SD=1.51) and super-matchers (M=2.63, SD=1.03) did not differ significantly (p= 0.414), and both groups had higher scores than matchers (M=1.86, SD=0.89; p's=0.013 and 0.046, respectively). That is, higher levels of positive symptoms were associated with more behavioral optimizing.

Because both positive and negative symptoms appear to be related to being classified as an optimizer, we examined the role of both symptom types in a binomial logistic regression analysis. In this approach, the specific contribution of each symptom type is examined using standardized BPRS and SANS scores, and yields a test for statistical significance, as well as an odds ratio and a confidence interval that is more easily interpreted. That is, one can determine how much influence a one standard deviation increase in either BPRS positive or SANS negative symptoms makes in establishing the classification as an optimizer versus all other behavior patterns. The results suggest that the odds of being an optimizer increase with negative symptoms [Exp(B)=3.918, CI=1.207-12.722, *p*= 0.023], whereas the effect of positive symptoms is not significant [Exp(B)=2.024, CI=0.803-5.101, *p*= 0.135]. This essentially means that with each one standard deviation increase in negative symptom severity, the chances of being classified as an optimizer increases by almost 80%.

We also examined whether performance on the WASI, WTAR, WRAT and MATRICS battery composite score differed among patients meeting the various response strategy classifications. As seen in Table 2, no significant differences in performance on these neuropsychological measures emerged among the three response strategy groups for either patients or controls. It is noteworthy that, despite having higher levels of positive and negative symptoms, the patient optimizers performed best on cognitive tests reflecting premorbid functioning (WTAR, WRAT) and were also found to have the highest current IQ score as assessed by the WASI (M=111.86, SD=12.13), when compared to matchers (M=93.54, SD=12.84) and super-matchers (M=101.33, SD=15.87).

Table 2. Demographic information and cognitive assessments for performance groups

Measure	Matchers M (SD)	Super-matchers M (SD)	Optimizers M (SD)	F / χ2	p-value
Age	44.48 (9.44)	44.97 (9.09)	43.81 (10.19)	0.033	0.967
Education (years)	12.67 (2.70)	13.08 (2.02)	13.14 (2.04)	0.173	0.841
Paternal Education (years)	13.32 (3.88)	14.45 (4.41)	13.71 (3.15)	0.292	0.749
Gender (M: F)	13:11	9:03	7:00	5.618	0.444
Race				1.623	0.579
African American	9	5	1		
Caucasian	14	7	5		
Other	1	0	1		
Standard Neuropsychology					
WRAT	90.26 (13.68)	93.08 (18.54)	101.43 (8.98)	1.559	0.223
WTAR	90.83 (17.26)	94.67 (20.82)	106.29 (12.41)	2.066	0.14
WASI	93.54 (12.84)	101.33 (15.87)	111.86 (12.13)	1.819	0.175
MATRICS battery composite score	31.96 (14.63)	30.92 (16.93)	29.71 (8.80)	0.069	0.934
Clinical Ratings					
BPRS Total	32.44 (6.38)	36.83 (6.74)	43.86 (12.68)	5.998	0.005
BPRS Positive	1.86 (0.88)	2.63 (1.03)	3.04 (1.51)	4.352	0.020
BPRS Negative	1.64 (0.66)	1.56 (0.61)	2.36 (0.92)	3.381	0.044
BPRS Disorganized	1.24 (0.40)	1.55 (0.48)	1.46 (0.74)	1.690	0.198
SANS Total	27.87 (8.49)	19.67 (11.67)	39.14 (15.41)	7.305	0.002
Calgary Depression Scale	1.74 (1.69)	2.08 (2.35)	3.71 (3.95)	1.894	0.164
Antipsychotic Medication Regimen					
Haloperidol or Fluphenazine only	1	0	0		
Clozapine only	10	4	4		
Other second-generation only	10	6	1		
Clozapine + another antipsychotic	3	2	2		
First -generation + second-generation antipsychotic	0	0	1		
CONTROLS					
Age	46.67 (9.86)	43.01 (10.46)	41.34 (11.26)	0.597	0.558
Education (years)	15.50 (1.96)	15.10 (2.23)	13.71 (1.80)	1.680	0.208
Paternal Education (years)	13.80 (3.23)	11.80 (4.19)	12.17 (2.56)	0.885	0.426
Gender (M: F)	7:03	6:04	6:01		
Race					
African American	2	4	4		
Caucasian	8	6	3		
Other	0	0	0		
Standard Neuropsychology					
WRAT	104.50 (15.82)	97.80 (17.11)	96.14 (19.93)	0.585	0.565
WTAR	107.20 (12.84)	99.90 (19.26)	100.14 (21.51)	0.516	0.603
WASI	117.50 (10.47)	108.70 (12.58)	110.71 (17.16)	1.197	0.320
MATRICS battery composite score	55.56 (12.31)	42.78 (14.85)	46.86 (19.13)	1.620	0.221

DISCUSSION

Consistent with numerous studies from the literature on reward learning in healthy individuals, we observed that, when presented with a two-choice probability learning task, the majority of subjects show suboptimal levels of performance in that they allocate choices according to their relative expected values (in this case, 70% and 30%). In examining variability in performance based on the underlying clinical characteristics of patients with schizophrenia, we found that the most adaptive and profitable strategy was adopted by a subgroup of patients exhibiting the most severe negative and positive symptoms. That is, the patients who chose the optimal side on nearly all of the trials exhibited higher levels of negative symptoms than the ones who matched or super-matched the probabilities of reinforcement. Although we observed that patients who showed behavioral optimizing in this task also exhibited the greatest positive symptoms, we found that the severity of negative rather than positive symptoms had an impact on predicting which behavior pattern a patient will adopt.

Importantly, the group of patients showing optimal behavior did not differ significantly from patients in other performance classifications in age, educational level, parental educational level, or racial/gender make-up. Furthermore, subgroups of patients identified by task performance did not differ in disease duration, medication status, or measures of neuropsychological functioning, such as working memory capacity, speed of processing, or hypothesis testing. Thus, it seems unlikely that reward maximization was the result of general intellectual impairment. One potential explanation for these results is that patients classified as optimizers have a tendency toward reduced exploration of response options under conditions of uncertainty. Supporting this interpretation are recent computational modeling results from our group, which indicate that higher levels of negative symptoms are associated with reduced uncertainty-driven exploration on a behavioral reward learning task (8). In the current task environment, patients with the highest ratings for negative symptoms showed a reduced tendency to explore response alternatives defined by spatial locations. Based on additional work from our group (27), we suspect that this reduced tendency to explore response alternatives would extend to tasks in which optimal responses are defined by other stimulus features.

We acknowledge that, in static environments such as those in the current task, it is plausible to expect that a tendency toward reduced exploration could lead to significantly fewer shifts from the optimal response and therefore result in earning a higher percentage of rewards. That is, sticking to a winning choice may make sense, provided that the subject is certain that the reward frequencies and magnitudes are, and will remain, constant. Such behavior would be less than optimal if the previous poor choice had changed in value and was now more desirable. Thus, consistently selecting a previously-rewarding choice comes at the cost of not knowing whether the environment has changed and potentially leading an individual to avoid certain responses.

Such a learning pattern could be considered a viable contributor to symptoms of avolition and reduced reward seeking in people with schizophrenia.

Numerous brain systems have been linked to exploratory behavior, including the dopaminergic, cholinergic, and noradrenergic systems, especially through their targets in the prefrontal cortex (PFC) (32, 33, 35). Importantly, PFC is known to play a critical role in tracking reinforcement, computing its magnitude and representing value (36, 37). Furthermore, recent neuroimaging work (38) suggests that probability matching relies on PFC function, possibly in the service of explicitly representing reinforcement histories. If probability matching is, in fact, a phenomenon caused by PFC-dependent feedback sensitivity, we would expect patients with schizophrenia to perform poorly in such environments. The association of negative symptoms with decreased sensitivity to feedback could result in less outcome-driven and more internally-generated behavior. Using a similar two choice guessing task, Paulus and colleagues (39, 40) have demonstrated that long histories of previous responses (aside from external cues) exert a greater influence on the choices of individuals with schizophrenia relative to normal controls. In our study, patients with the most severe negative symptoms also may have been driven inordinately by response histories and habits, rather than recent feedback.

Paradoxically, our task environment provided an opportunity for the failure to engage in meaningful exploration and relative insensitivity to probabilistic, and occasionally misleading, feedback to be advantageous and result in maximum payoff. However, in volatile, non-stationary learning environments, as most real-world environments are, this quality would be extremely maladaptive, and would clinically manifest in a very limited, perseverative behavioral repertoire, where responses are not sensitive to changes in context. One would expect that the inevitable failures that would occur would lead to further withdrawal from novel or challenging situations and result in a form of inertia. Although our findings can be interpreted in a way that supports this notion, our task design did not allow us to study the effect of the volatility of the environment on exploratory behavior since the respective probabilities as well as the magnitude of reinforcement were kept constant. The impact of symptoms on a patient's tendency to exploit versus explore in non-stationary environments is the subject of ongoing work in our group.

The fact that our group-wise analyses found no significant differences in global percentage of trials on which the optimal choice is made or learning rate between patient and normal controls should not be seen as evidence of intact reinforcement learning in schizophrenia: the current task was very simple and there is reliable evidence of impairment when more challenging tasks are used (5, 8). The current data suggest that a more fine-grained understanding of reward processing and learning deficits may be obtained by dividing the larger population of patients with schizophrenia into more homogeneous subgroups, possibly using variables such as negative symptoms severity as a classifying factor. Given the heterogeneity of the illness, it

appears highly likely that the mapping from cognitive process to neural mechanism to behavior will be much more successful using a symptomatic endpoint rather than a broad diagnostic class.

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Chapter 4

From affective experience to motivated action: tracking reward-seeking and punishment-avoidant behavior in real-life

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ABSTRACT

Many of the decisions and actions in everyday life result from implicit learning processes. These processes likely play an important role in the development of psychopathology. This study examines whether reported behavior can be traced to previously experienced associations between behavior and the accompanying affective experience. 621 female individuals participated in an Experience Sampling Method (ESM) data collection. Measures of affect, daily life experience and behavior were collected at 10 semi-random moments of the day for 5 consecutive days. It was examined whether affective experience that was paired with certain behavior at previous measurements modified the likelihood to show similar behavior at next measurement moments. This was assessed for two relevant behaviors: physical activity and social context. Multilevel time-lagged analyses were used for moderation analyses where behavior at (t-2 and t-1) was used as a predictor for similar behavior at (t). It was hypothesized that positive affect associated with behavior at (t-2 and t-1) would augment the likelihood of similar behavior at (t) (reward-seeking behavior) whereas negative affect would reduce it (punishment-avoidant behavior). Analyses were performed both at the level of observations (a time scale with units of ± 90 min) and day level (a time scale with units of 24 h). Affect valence indeed moderated the extent to which previous behavior predicted similar behavior later in time, at both day- and beep-level. This study showed for the first time that it is feasible to track reward-seeking and punishment-avoidant behavior prospectively in humans in the flow of daily life. This opens up a new toolbox to examine processes determining goal-oriented behavior in relation to psychopathology in humans.

INTRODUCTION

Many of the decisions and actions in everyday life result from learning processes that are guided by interaction with environmental cues. The capacity to form and continuously update internal representations of stimulus-response associations allows one to select the most advantageous response from a repertoire, and to accurately predict its outcome (1-3). These implicit associative learning processes are therefore critical mediators of daily life decisions and future course of actions. Consider the example of Peter and Christine who both join the athletics club, in Peter's case with the goal of becoming a long-distance runner and in Christine's to make new friends. Peter is tall, slim and fit which predisposes him to excel in endurance activities. He enjoys the training sessions, does not experience a lot of unpleasant physical sensations and improves quickly, soon becoming one of the best runners in the group. As a result, Peter's repeated exposures to positive outcomes motivate him to keep training. Christine, on the other hand, who is a little shy, sensitive and less interested in running, welcomes the opportunity to join her new teammates in a party. She enjoys the party until one of the girls makes a critical remark about her clothes. She feels very awkward goes home early and skips some practices. When she is invited again to a similar party by her teammates, she declines, until eventually quitting the team. Although seemingly very different, both Peter's and Christine's behavior was governed by two essential mechanisms of operant conditioning (3-6). Positive reinforcement learning is the process through which new stimuli acquire motivational salience by virtue of being associated with positive emotions, thus becoming rewards. Rewards have, by definition, the potential to increase the likelihood that an organism will engage in actions previously associated with positive affective state (6), as demonstrated by Peter's case. Punishment learning, on the other hand, is necessary to avoid aversive outcomes (5, 7). Aversive stimuli decrease the frequency of behavior linked to negative affective states (6), such as those in Christine's situation. In both examples the learned associations between (un)pleasant affective experiences and the individual's behavior play an important role in motivating future choices to maintain or extinguish the pertinent behavior. Associative learning processes, therefore, allow for a flexible adaptation of behavior by guiding organisms in decisions of approach and avoidance of daily life situations, and ultimately prompt and maintain goal-oriented behavior (6). The fact that the neural circuits governing associative learning are present even in organisms with the most primitive neural networks (8) demonstrates the importance of this mechanism in survival and evolution.

Experimental studies have shown evidence for positive and negative experiences modifying behavior in humans (5, 9-13). For example, positive self-talk and self-esteem could be increased by pairing images of a person's own body with positive stimuli that signal social acceptance (11). In addition, the unconscious tendency to consume alcohol was found to decrease following pairing of the alcohol cue with an aversive out-

come (9). Importantly, inadequate reward and punishment learning may play a key role in many forms of deviant behavior (3, 5, 14). For example, whereas impaired punishment learning was shown to be associated with increased risk-taking behavior and gambling (15), enhanced punishment-avoidance is thought to be related to the acquisition of pain-related fear (12) and in the withdrawal from social situations. Similarly, abnormally high tendency for reward-seeking behavior is linked with the risk for developing an addiction (9) and eating disorders (16), while suboptimal reward-driven behavior is believed to be associated with anhedonia, avolition and depression (17, 18).

Despite the importance of reward and punishment learning in human behavior and their putative relevance to mental health, to date, incentive-driven responding has not been studied prospectively in the flow of daily life. This endeavour would provide an ecologically valid assessment of the extent to which affective experience motivates action, with the potential of uncovering real-life behavioral patterns linked to compromised mental health.

Momentary assessment techniques (19-27), enabling the researcher to capture the film rather than a snapshot of daily life reality (28-31), are ideally suited to examine the subtle temporal associations between affect, daily life situations over the course of the day(s). Therefore, we can use this method to detect patterns of reward-seeking and punishment-avoidant behavior by examining whether rewarding or punishing affective states occurring in a certain daily life context, modify the likelihood of engaging in similar daily life contexts in the near future. If we can detect patterns in the data showing that rewarding or punishing experiences influence the likelihood that similar behavior is repeated, then this would support the idea that we can detect the associative learning processes that motivate future behavior.

Aims of the study

The aim of this study is to examine whether it is possible to prospectively track the propagation of reward and punishment-driven behavior in humans using momentary assessment methodology. In order to obtain initial proof-of-principle, this study focuses on two frequently occurring daily life situations which are relevant to mental health states: social context and physical activity. To this end, data pertaining to a general population sample of 621 individuals who participated in an Experience Sampling Method (ESM) study were analysed.

METHODS

Sample

The study sample consisted of 621 female subjects from the general population, who were part of twin pairs or were sisters of twin pairs living in Flanders (Belgium). Their age was between 18 and 46 years. Of the 621 subjects, 610 participated in the ESM study. Most participants were recruited from the East-Flanders Prospective Twin Survey, a population-based survey prospectively recording all multiple births in the province of East-Flanders since 1964 (32, 33). Selection criteria were being female and being over 18 years of age. Participants received a financial compensation for participation of 500 Belgium Francs (an equivalent of 12,5 euros). Although subjects were twins, the current hypothesis did not require twin methodology. The project was approved by the Medical Ethics Committee of the K.U. Leuven under the number B3222010766. All participants gave written informed consent. Originally, the study was designed to assess stress-sensitivity in daily life; taking into consideration evidence for qualitative differences in the type of environmental stressors that are associated with depression in men and women (34, 35), the sample was female only. Mean age of the participants was 27 years (SD: 7.6 years, range (18-46). Sixty-five percent had a college or university degree, 33% completed secondary education and 2% had primary education only. The majority was currently employed (61.3% employed, 33.8% student, 2.2% unemployed, 2.3% homemaker and 0.4% sick leave).

Experience Sampling Method

The experience sampling method (ESM) was used to conduct momentary assessments on 5 consecutive days. Subjects received a digital wristwatch and a set of ESM self-assessment forms collated in a booklet for each day. The wristwatch was programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-minute time blocks between 7:30 and 22:30. The study uses a semi-random beep design in order to prevent anticipatory behavior of participants, but also used the constraint that no two signals could occur within 15 minutes of each other(36). Beep intervals did not overlap, thus ensuring that the first beep of the day was always earlier in time than the second and the second earlier than the third etc. The semi-random beep design was set up in a way that every moment of the day had an equal likelihood of being sampled.

Participants were aware that 10 beep signals would occur in a day between 7.30 and 22.30. They were instructed to fill out the ESM diary directly after each beep signal. The diary consisted of short questions on the current affect, behavior and appraisals of the current situation (see below). All self-assessments were rated on 7-point Likert scales. Trained research assistants with ample experience in momentary assessment technology explained the ESM procedure to the participants during an initial

briefing session, and a practice form was completed to confirm that subjects were able to understand the 7-point Likert scale. Subjects could call a telephone number in case they had questions or problems during the ESM sampling period. Subjects were instructed to complete their reports immediately after the beep, thus minimizing memory distortion, and to record the time at which they completed the form. In order to know whether the subjects had completed the form within 15 min of the beep, the time at which subjects indicated they completed the report was compared to the actual time of the beep. All reports not filled in within 15 min after the beep were excluded from the analysis, since previous work (22) has shown that reports completed after this interval are less reliable and consequently less valid. In addition, subjects with less than 17 valid reports (out of 50) were excluded from the analysis, as previous work has shown that measures of individuals with less than 30% of completed reports are less reliable (22). Compliance to the ESM protocol was very high (96.4%) (37).

Measurements

Momentary affect

Momentary affective states were assessed with 15 adjectives rated on 7-point likert scales. The choice of the ESM affect items was guided in part by the PANAS questionnaire (38) and in part by the results of previous ESM studies (selecting items with high loadings on NA and PA latent factors and sufficient within-person variability). A Factor analysis on the affect items identified two affect factors with eigenvalue >1. Ratings on the items 'insecure', 'lonely', 'anxious', 'low', 'guilty' and 'suspicious' –weighted for factor loadings– were averaged to form NA (respective loadings were: 0.71, 0.60, 0.66, 0.68, 0.61, 0.64). The weighted average of ratings on 'cheerful', 'content', 'energetic', 'enthusiastic' formed PA (respective loadings were: 0.84, 0.71, 0.84, 0.83). The strongest cross-loading was -0.13 (loading of 'content' on NA factor). Five affect items did not load specifically on one of these factors. These were 'tired', 'relaxed', 'irritated', 'sad' and 'calm'. Conform previous publications (39, 40) that used this sample, these five items were not used in the variables that measure NA and PA.

Appraisal of social context

Appraisal of the social context was assessed by asking participants whether or not they were alone at the time of the beep. If not alone, they were asked whether they liked the company they were in at that moment. This was rated on a 7-point likert scale (from 'not at all' (1) to 'very much' (7)).

Physical activity

ESM physical activity was assessed with a single item asking subjects how physically active they had been since the last beep, rated on a 7-point likert scale. During the

ESM briefing, participants were instructed on how to score their level of physical activity. To this end, they were given an indication of the level of activity that corresponded to the score on the 7-point likert scale. Examples were provided as anchor points as follows: a score of '1' corresponds to the level of physical activity of 'resting'; '2' corresponds to 'sitting'; '3' to 'walking'; '4' to 'household chores such as vacuum cleaning'; '5' to 'biking'; '6' to 'playing tennis' and '7' to 'running'. These anchor points were also provided in the ESM self-assessment forms that subjects completed at each beep. For instance, for each physical activity approximately equalling the level of physical activity associated with vacuum cleaning, participants rated a score of 4 on the 7-point likert scale.

Statistical analysis

ESM data have a hierarchical structure in which ESM observations (level 1) are clustered within participants (level 2). STATA 11.0(41) was used to conduct multilevel regression analyses. Time-lagged multilevel analyses were used to examine reward- and punishment-based behavior prospectively over time.

Reward-related behavior is the result of an associative process in which positive affective experience is paired with a behavior, and the frequency of this behavior increases. Assuming that positive affect is rewarding, reward-driven behavior was operationalized in ESM as the moderating effect of PA at (t-1) on the probability that behavior at (t-1) was repeated at (t) (figure 1). Punishment-related behavior represents the opposite process in which the occurrence of the behavior previously associated with negative affective experience decreases. Assuming that negative affect is punishing, punishment-related behavior was thus operationalized in ESM as the moderating effect of NA at (t-1) on the probability that behavior at (t-1) was repeated at (t) (figure 1). Mathematically, the i-th momentary observation score of subject j is modelled as follows:

$\text{Behavior}_{ij\ t} = \beta_0 + \beta_1\ \text{affect}_{ij\ t-1} + \beta_2\ \text{behavior}_{ij\ t-1} + \beta_3\ \text{affect}_{ij\ t-1}*\text{behavior}_{ij\ t-1} + \zeta_j + \varepsilon_{ij.}$

Here, ζ_j represents the subject's deviation from the overall mean (random intercept).

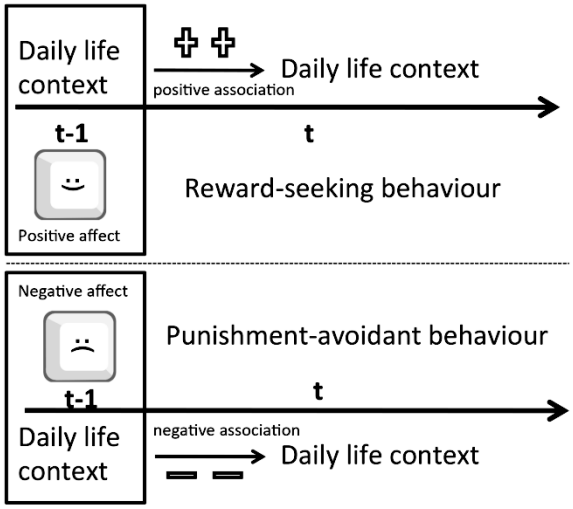
In order to obtain a regression model that purely tests within-subject effects, the between-subject regression was subtracted from the original regression model, as follows: $\text{Behavior}_{ij\ t}\ \text{behavior}_{.j\ t} = \beta_1\ (\text{affect}_{ij\ t-1} - \text{affect}_{.j\ t-1}) + \beta_2\ (\text{behavior}_{ij\ t-1} - \text{behavior}_{.j\ t-1}) + \beta_3\ (\text{affect}_{ij\ t-1}*\text{behavior}_{ij\ t-1} - \text{affect}_{.j\ t-1}*\text{behavior}_{.j\ t-1}) + \varepsilon_{ij} - \varepsilon_{.j.}$ This was performed using the XTREG command combined with the fixed effects (*fe)* option. Since the *fe* option is available only for multilevel analyses with no more than one extra level, clustering within twin pairs was accounted for by adding twin pair id as a covariate. The above example equations describe the model including one lag effect. The regressions performed in this study included 2 lags.

Generalisability over different time frames: In addition to the beep-level (t duration= ± 90 min), reward-related and punishment-related behaviors were also explored

at day-level (t duration= 1 day). For the day-level analyses, beep moment results of affect, behavior and the interaction thereof were averaged per beep over the day. These analyses thus examined whether the average of the repeated (maximum 10) combinations of affect and behavior at day (t-1) impacted on the average behavior on beep moments at day (t).

Specificity of effects: Additionally, cross-context associations were added to examine the specificity of the effect If time-lagged effects across the different behavioral contexts are equally strong as those within behavioral contexts, this would be indicative for non-specific effects of affect impacting on future behavior in general. If, on the other hand, within-context effects are much stronger than cross-context effects, then this supports the idea that we detected behavior-specific processes of associative learning.

Figure 1: Schematic representation of the operational definition of reward- and punishment-related behavior



Reward-related behavior is conceptualized as the daily life behavior (such as being in pleasant company or engaging in physical activity) that increases in frequency at time t upon being associated with positive affect at time t-1. Punishment-related behavior is conceptualized as the daily life behavior the frequency of which decreases at time t upon being associated with negative affect at time t-1. Time lags t-1 and t-2 are examined in this study where t represents the time lag of one beep moment (± 90 minutes) or the time lag of one day.

RESULTS

Of the 610 subjects who participated in the ESM study, 31 were excluded because they had too few (less than 30%) beeps with valid responses. Of the 579 remaining subjects, 12 participants had been part of the first pilot data collection. Some of the PA items

that were present in the final diary were not present in the pilot diary. As information for some of the PA items was not available the total PA scores could not be computed for these 12 participants. Another 4 participants had no intra-individual variations in or no consecutive measurement moments for physical activity, and were therefore excluded from the analysis. In the various analyses, the total number of beep-level measurements varied between 9,990 and 12,228. The total number of day-level measurements varied between 1,642 and 1,693. In table 1 the correlations at observation-level are shown between NA, PA, (un)pleasantness of company and physical activity.

Table 1: Correlations (at observation-level) between negative affect, positive affect, (un)pleasantness of company and physical activity.

	Negative affect	Positive affect	(Un)pleasantness of company	Physical activity
Negative affect	-			
Positive affect	-0,35	-		
(Un)pleasantness of company	-0,21	0,30	-	
Physical activity	0,007	0,11	-0,02	-

Reward-related behavior

Beep-level

Table 2 shows the regression analyses results regarding temporal patterns of reward-related behavior for beep and day-level. Significant within-context interaction effects were found for both behaviors: engaging in physical activity and being in pleasant company. More specifically, PA moderated the effect of physical activity at beep (t-1) on physical activity at beep (t). The same was true for being in pleasant company. Significant effects were observed only for associations over one lag. No significant cross-context effects (e.g. from physical activity to pleasant company or vice versa) were found (see table 2 and figure 2).

Table 2: Reward-related behavior: Regression coefficients and p-values of the *interaction effects* (PA x behavior at lags 1 and 2 on behavior at (t)) at beep and day level.

BEEP-LEVEL	Lag 1		Lag 2	
Within-context effects	*b-coefficient*	*p-value*	*b-coefficient*	*p-value*
PA_{t-n}[Φ] x pleasant $company_{t-n}$ on pleasant $company_t$	0.016	0.037*	0.008	0.287
PA_{t-n} x physical $activity_{t-n}$ on physical $activity_t$	0.022	0.012*	0.002	0.808
Cross-context effects				
PA_{t-n} x pleasant $company_{t-n}$ on physical $activity_t$	0.003	0.678	0.001	0.906
PA_{t-n} x physical $activity_{t-n}$ on pleasant $company_t$	0.009	0.422	-0.012	0.279
DAY-LEVEL				
Within-context effects	*b-coefficient*	*p-value*	*b-coefficient*	*p-value*
(PA x pleasant company)$_{t-n}$ on pleasant $company_t$	0.091	<0.001*	0.029	0.247
(PA x physical activity)$_{t-n}$ on physical $activity_t$	-0.014	0.528	0.023	0.288
Cross-context effects				
(PA x pleasant company)$_{t-n}$ on physical $activity_t$	-0.048	0.025*	-0.062	0.004*
(PA x physical activity)$_{t-n}$ on pleasant $company_t$	-0.057	0.053	-0.086	0.003*

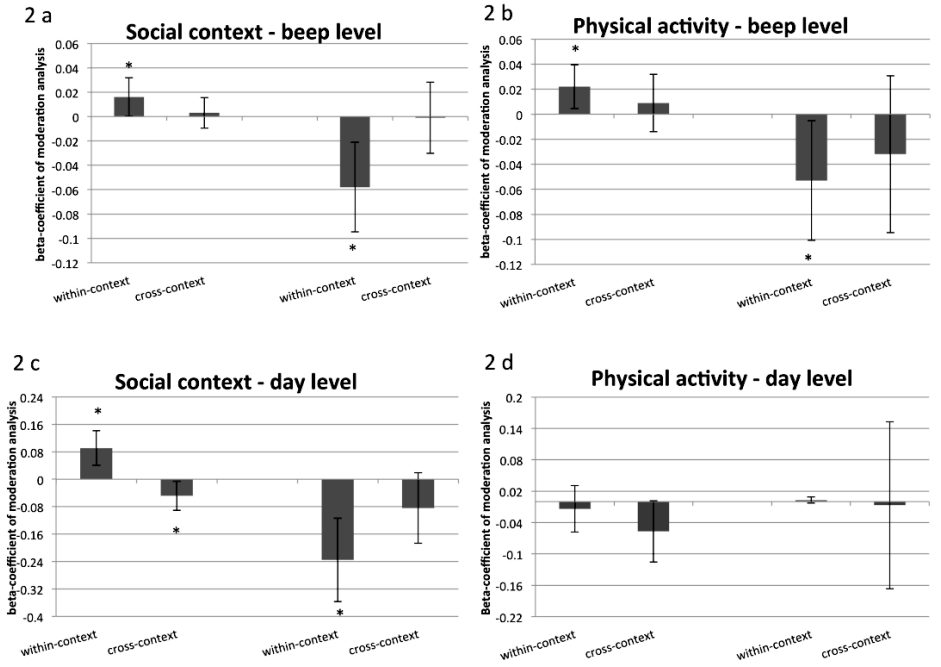
[Φ]n is either 1 (in case of 1 lag) or 2 (in case of 2 lags)
* significant finding ($\alpha < 0.05$)

Day-level

PA at day (t-1) moderated the effect of being in pleasant company at day t-1 on being in pleasant company at day (t) (one lag). This effect was not found for day (t-2) on day (t)(two lags). Thus, higher levels of PA in the context of pleasant company increased the level of pleasant company on the next day. For physical activity, no significant interaction effects were found for lag 1 and 2 (see table 2; figure 2). However, strongly negative *main effects* were observed regarding physical activity at day (t-1) and day (t-2) on day (t) (B=-0.28, p<0.001 and B=-0.24, p<0.001, respectively). This suggests a sinusoid pattern in which high levels of physical activity on one day are associated with less activity the next two days. Therefore, more lags might be necessary to reveal a potentially delayed effect of PA during physical activity and the occurrence of physical activity in the future. In order to examine such pattern, a *post-hoc* test was carried out of the effects of an additional lag. Corrected for effects at lag 1 and 2, lag 3 revealed a significant interaction effect where PA positively moderated the effect of physical activity at day (t-3) on physical activity at day (t) (B=0.106, p=0.003; figure 3). No posi-

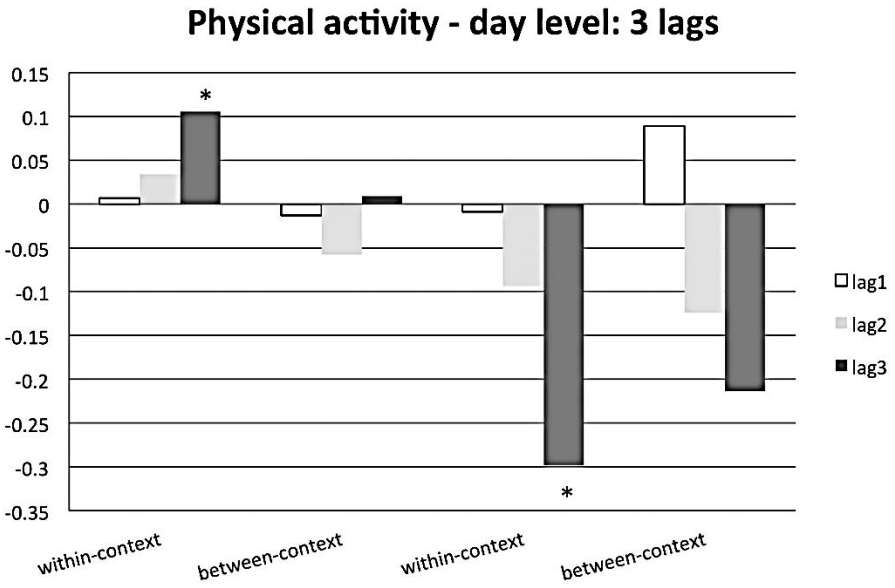
tive moderation effects of PA were found in cross-context analyses. In contrast, negative moderation was observed (see table 2).

Figure 2: Beta-coefficients for reward- and punishment-related behavior at beep level and day level for lag t-1



The first two bars in each figure represent the analyses with positive affect at t-1 as the moderator of the time-lagged association between daily contexts and the second two bars represent the analyses with negative affect at t-1 as the moderator of these analyses. Each first bar is the within-context association (the same context is predictor at t-1 and outcome measure at t). This association is hypothesized to reflect reward-seeking or punishment-avoidant behavior based on previous pairing of that context with positive or negative affect, respectively. The second bar refers to the cross-context association: the extent to which affect moderates the time-lagged association across daily contexts (for example: social context as the predictor and physical activity as the outcome). Error bars represent confidence intervals.

Figure 3: Beta-coefficients of reward- and punishment-based responding for physical activity for three lag moments (t-1, t-2 and t-3)



A stronger association of physical activity with positive affect at day t-3, corrected for effects at day t-2 and day t-1, increases physical activity at day t. Similarly, a stronger association between physical activity with negative affect at day t-2, corrected for effects at day t-2 and day t-1, decreases physical activity at day t. No significant cross-context effects were found. Error bars represent confidence intervals.

Punishment-related behavior

Beep-level

NA at beep (t-1) negatively moderated the effect of unpleasant company at beep (t-1) on unpleasant company at beep (t). Thus, higher levels of NA decreased the effect of being in unpleasant company at beep (t-1) on being in unpleasant company at beep (t). No interaction effect of beep (t-2) was observed. A similar effect was found for physical activity in that NA at beep (t-1) negatively moderated the effect of physical activity at beep (t-1) on physical activity at beep (t). No significant negative moderation was observed for cross-context interaction effects (see table 3 for effect sizes and p-values of the interaction effects).

Table 3: Punishment-related behavior: Regression coefficients and p-values of the *interaction effects* (NA x behavior at lags 1 and 2 on behavior at (t)) at beep and day level.

	Lag 1		Lag 2	
BEEP-LEVEL				
Within-context effects	*b-coefficient*	*p-value*	*b-coefficient*	*p-value*
NA_{t-n}^{ϕ} x unpleasant $company_{t-n}$ on unpleasant $company_t$	-0.058	0.002*	0.004	0.812
NA_{t-n} x physical $activity_{t-n}$ on physical $activity_t$	-0.053	0.027*	-0.019	0.409
Cross-context effects				
NA_{t-n} x unpleasant $company_{t-n}$ on physical $activity_t$	-0.001	0.969	-0.023	0.115
NA_{t-n} x physical $activity_{t-n}$ on unpleasant $company_t$	-0.032	0.314	-0.004	0.900
DAY-LEVEL				
Within-context effects	*b-coefficient*	*p-value*	*b-coefficient*	*p-value*
(NA x unpleasant $company)_{t-n}$ on unpleasant $company_t$	-0.235	<0.001	0.005	0.941
(NA x physical $activity)_{t-n}$ on physical $activity_t$	0.003	0.960	-0.012	0.849
Cross-context effects				
(NA x unpleasant $company)_{t-n}$ on physical $activity_t$	-0.084	0.101	-0.001	0.983
(NA x physical $activity)_{t-n}$ on unpleasant $company_t$	-0.007	0.931	-0.115	0.172

$^{\phi}$n is either 1 (in case of 1 lag) or 2 (in case of 2 lags)

* significant finding ($\alpha < 0.05$)

Day-level

NA at day (t-1) at moments of unpleasant company at day (t-1) negatively moderated the effect of being in unpleasant company at day (t-1) on being in unpleasant company at day (t). This interaction effect was not found for day (t-2). No significant negative moderation was observed for physical activity (see table 3). However, similar to the analyses of reward-related behavior, there were significant negative main effects of physical activity at day (t-1) on physical activity at day (t), possibly obscuring the associative patterns. Therefore, the effect of an extra day-lag was examined *post-hoc*. Results showed that NA negatively moderated the effect of physical activity at day (t-3) on physical activity at day (t) (B=-0.298, p=0.008 (see figure 3). No significant cross-context interaction effects were found (see table 3).

DISCUSSION

The hypothesis that subtle prospective patterns of the propagation of reward and punishment-related behavior can be detected using momentary assessment techniques was confirmed. Both at beep and day level, affective experience associated with certain behavioral contexts influenced the frequency of future occurrence of similar behavioral contexts in daily life.

Social context

As expected, the experience of positive affect in pleasant social contexts increased the extent to which these contexts predicted the occurrence of similar social contexts in the near future at beep and day level (within-context), but did not increase the extent to which pleasant social contexts predicted the occurrence of other activities, such as physical activity (cross-context). At beep level, where measurements are on average 90 minutes apart, this finding could indicate two things. First, it could reflect a tendency to search for new enjoyable company directly following a previous rewarding social experience. Second, the finding may indicate that people tend to prolong the amount of time in a specific social context if it is associated with experience of positive affect. At day level, the latter interpretation is not very likely. Here, the repeated associations between positive affect and pleasant social contexts, derived from all measurement moments during the day together, predicted the frequency of being in similar pleasant company the next day. Day level findings, therefore, might reflect associative processes that involve a more complex integration of multiple response-outcome contingencies to effectively evaluate what future actions may be beneficial in the long-term. It is thought that higher-order cortical processing in the prefrontal and anterior cingulated cortex is involved in decision-making based on the synthesis and evaluation of a large number of previously internalized associations (42-45). The beep-level effects in this study could be more indicative of short-term responses to immediate affective experience instead of being based on the integration of a long-term history of experiences.

Also, as predicted, negative affect associated with unpleasant social contexts decreased the likelihood of the occurrence of similar social contexts in the near future at both beep- and day-level. Similar to the reasoning above, the beep-level findings could mean either that people avoided new negative social situations after negative social experiences one or two hours earlier that day, or that people tended to shorten their contact with company in which they experienced negative affect. In conclusion, the valence of affective experience associated with social behavior was found to modify the frequency of occurrence of this behavior. This effect, however, was detected for one lag, but not for two lags. This means that the pertinent affective state was found to modify behavior for up to 90 minutes at beep-level and for up to 1 day at day-level.

Physical activity

As hypothesized, significant within-context and non-significant cross-context effects were observed for physical activity at beep-level. That is, positive affective experience in the context of physical activity predicted increased frequency of occurrence of physical activity at the next measurement moment. Similarly, negative affective experience in the context of physical activity was associated with decreased propensity to engage in physical activity at the next measurement moment. Thus, both reward and punishment contingencies were found to modify behavior at beep-level. Similar to social context, these effects were only found to hold at one time lag but not at two time lags. In contrast to the social context findings, however, no significant association between affective experience related to physical activity and frequency of future engagement in physical activity was detected at day level. The lack of significant findings at day level is likely caused by the strongly negative association between physical activity on similar activity the next day. Arguably, this pattern may be a demonstration of the natural day-to-day fluctuations in physical activity levels; people tend to be less active during the days following a physically more challenging day, reflecting a compensatory mechanism. It can be hypothesised that these day-to-day fluctuations in activity obscured the effects of the propagation of associative processes, since adding an additional third lag (figure 3) revealed the expected within- and cross-context effects. It is thus important to find the appropriate level at which associative processes can be modelled for each type of behavior studied. Behavioral modification regarding physical activity apparently requires examining patterns over multiple days to accurately track the intermittent nature of physical activity and rest.

Experimental paradigms

Previously, studies have shown evidence of reward and punishment-driven behavior using experimental paradigms involving monetary rewards or losses. One experiment showed, using a monetary incentive delay task, that the ability to learn to avoid losses was associated with differences in insular sensitivity to anticipated losses and with self-reported levels of anxiety (46). Another study showed that higher arousal responses at moments of a threat cue -signalling the delivery of a shock- were associated with increased learning of avoidance responses (10). Also, reward sensitivity assessed experimentally (18, 43, 47-53) using monetary incentives was found to be modulated by stress, genetic predisposition and anhedonic states. To date, however, it is unknown how experimental findings based on secondary rewards and punishments extrapolate to real-life stimulus-response relationships, and how these manifest themselves in real-life situations, such as social contexts or personal activities. While experimental studies provide valuable insights into psychological and biological mechanisms of relevant traits, momentary assessment techniques may be able to expand on their findings and provide real-life validity to experimental associative processing models. Real life

validation of experimental findings would connect existing knowledge on mechanisms of reward- and punishment-driven responding with concrete patterns of daily life sub-optimal goal-oriented behavior that can be targeted for therapeutic modification. Therefore, future studies should combine experimental and momentary assessment techniques to examine how experimental findings translate to real life behavioral and affective patterns. To our knowledge, only one recent study (54) has combined experimental imaging and momentary assessment techniques. The authors showed that prefrontal stress-related dopamine activity is associated with real life reactivity to stress. The current momentary assessment study is the first to show that the propagation of incentive-based behavior can be examined in the flow of daily life, paving the way for the first study combining experimental and momentary *associative processing* paradigms.

Clinical relevance

The findings of the current study showed that behavior can be modified as a function of the affective experience associated with it, a pattern that was detectable at the micro-level with time lags of approximately 90 minutes, but also at day-level, with time lags of one day. It still needs to be established, though, whether these real-life associative patterns are indeed relevant to psychopathology, and whether their relevance can be modulated by time frame of analysis (beep or day) level. In other words, with respect to psychopathology, it remains to be determined whether it has potential to examine person-specific patterns of behavioral modification on a moment-to-moment or on a day-to-day basis.

However, should these prospectively measured behavioral patterns be involved in the development of psychopathology, then analysing momentary assessment data may represent a new tool to provide person-tailored clinically useful information (30)., Person-specific analyses may reveal person-tailored results regarding reward-seeking and punishment-avoidant behavior in real life. Such person-specific insights into daily life learning mechanisms may be of direct clinical use as input for therapist-patient contacts (30, 55-57).

Methodological issues

Effect sizes as reported in the current study are small. However, it is generally observed that in ESM studies effect sizes are smaller than effects found using traditional research designs, since in ESM effects occur over very small periods of time. These effects represent averaged effects that happen each 90 minutes or each day, depending on the time frame. Cumulatively, these repeated effects seem influential enough to impact on mental health as has been shown in previous studies (58-60).

Second, it is unknown whether the appraisal of company reflects an individual's active choice to be in the company of certain people, or the subjective interpretation

of the social context. If the latter is true, then these findings may reflect an alteration in the *appraisal* of the company, rather than a change in behavior. This would mean that the previous day's PA experience during pleasant company would improve the appraisal of company during the next day. This alternative interpretation can mean two things: (i) it reflects non-specific effects of PA. However, since no cross-context effects of PA were found, this is not probable; (ii) it reflects reward-modulated interpretation of daily life context. Either form of reward-driven modification (at the behavioral level or at the level of interpretation of daily life contexts) is clinically relevant. The same line of reasoning can be applied to the analyses on aversive learning.

The data of the current study were collected from 1999 to 2002. No electronic ESM devices were available at that time. However, compliance to the research protocol was electronically monitored in a subgroup of the sample and was determined to be high (see for more details (37)).

The current study used ESM for 5 consecutive days. However, longer periods of sampling (2-3 weeks) are preferable, especially for the modelling at day-level. The development of electronic ESM devices which are less demanding for participants than the paper-and-pencil method, may facilitate extended sampling periods.

Another limitation is the use of self-report only. Although ESM is one of the most accurate ways to assess dynamics in various affect states - that may not be estimated reliably using objective measures - , physical activity could also be assessed using actigraphy. Since both self-report and objective measures contain certain level of noise, the measure of physical activity could be improved by using a combination of both assessment methods. Finally, the sample included females only. Therefore, results do not necessarily extrapolate to male individuals.

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On stress

Chapter 5

No evidence for attenuated stress-induced extrastriatal dopamine signaling in psychotic disorder

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ABSTRACT

Stress is an important risk factor in the etiology of psychotic disorder. Preclinical work has shown that stress primarily increases DA transmission in the frontal cortex. Given that DA-mediated hypofrontality is hypothesized to be a cardinal feature of psychotic disorder, stress-related extrastriatal DA release may be altered in psychotic disorder. Here we quantified for the first time stress-induced extrastriatal DA release and the spatial extent of extrastriatal DA release in individuals with non-affective psychotic disorder (NAPD). 12 healthy volunteers (HV) and 12 matched drug free NAPD patients underwent a single infusion [^{18}F]fallypride positron emission tomography scan during which they completed the control and stress condition of the Montreal Imaging Stress Task. HV and NAPD did not differ in stress-induced [^{18}F]fallypride displacement and the spatial extent of stress-induced [^{18}F]fallypride displacement in medial prefrontal cortex (mPFC) and temporal cortex (TC). In the whole sample, the spatial extent of stress-induced radioligand displacement in right ventro-mPFC, but not dorso-mPFC or TC, was positively associated with task-induced subjective stress. Psychotic symptoms during the scan or negative, positive and general subscales of the Positive and Negative Syndrome Scale (PANSS) were not associated with stress-induced [^{18}F]fallypride displacement nor the spatial extent of stress-induced [^{18}F]fallypride displacement in NAPD. Our results do not offer evidence for altered stress-induced extrastriatal DA signaling in NAPD, nor altered functional relevance. The implications of these findings for the role of the DA system in NAPD and stress processing are discussed.

INTRODUCTION

In the last decade, significant progress has been made in understanding the role of the dopamine (DA) system in the human stress response(1-3). Evidence has emerged showing that, at least in part, the stress response is facilitated by DA release in the striatum(1, 2, 4) and prefrontal cortex (PFC)(5, 6). Dopaminergic (DAergic) involvement in the stress response is particularly relevant for psychiatric disorders such as psychotic disorder(7), as evidence suggests that stress plays an important role in the onset of psychotic symptoms(8)(9) and DAergic abnormalities are a hallmark feature of psychotic disorder(10). Investigating stress-related DAergic activity in the context of psychotic disorder could thus provide new insights into the pathogenesis of the disorder.

Stress-induced DAergic activity in humans has been studied *in vivo* with positron emission tomography (PET), hinging on competition between radioligand binding and endogenous DA release(11). In these studies, DA release was assessed during a psychosocial evaluation paradigm(2) (for metabolic stress see(12, 13)). Whereas psychosocial stress in healthy volunteers (HV) produced modest and variable changes in striatal DA release(1, 2, 4, 14), the same stressor reliably increased DA release in the (associative) striatum of individuals across the far end of the psychosis continuum(1)(2, 4). Importantly, this suggests that the putative association between stress and psychotic disorder may be moderated by the DA system.

Preclinical work, however, has revealed that short-lived stressors consistently and *primarily* increase DAergic activity in the PFC analogue of the rodent(15, 16). Moreover, selective destruction of frontal DA neurons increases stress-related DA transmission in mesolimbic regions(16, 17), hinting at a key regulatory role for PFC DA transmission in the stress response. Because DA-mediated hypofrontality is hypothesized to be a cardinal feature of psychotic disorder(18, 19), this preclinical work indirectly suggests that the well-documented link between stress and psychotic disorder(20, 21) may be underlain by cortical DA function. More specifically, decreased cortical DA function may constitute a neurochemical feature of vulnerability to psychotic disorder and underlie increased behavioral stress-sensitivity(21).

In the only two human studies currently available, psychosocial stress in HV increased medial PFC (mPFC) DA release(6) and increased the area (i.e. spatial extent) of mPFC DA release(5) assessed with high-affinity $D_{2/3}$ binding ligand [^{18}F]fallypride(22). In an add-on sample of first-degree relatives of patients with psychotic disorder, Lataster and colleagues(3) showed that the spatial extent of stress-induced mPFC DA release decreased as a function of increased subjective stress. Although this latter finding hints at stress-related DA-mediated hypofrontality in the psychosis continuum, investigating stress-induced PFC DAergic activity in established psychotic disorder could further elucidate the role of this mechanism in the pathogenesis of the illness. To these aims, we investigated the effect of psychosocial stress on extrastriatal DA signaling in a sam-

ple of HV and medication-free individuals with a diagnosis of non-affective psychotic disorder (NAPD) using [^{18}F]fallypride PET.

However, measuring extrastriatal DAergic activity remains methodologically challenging; the density of extrastriatal D_2 receptors is 2-8% compared to the striatum(23). Radioligands with suboptimal affinity and selectivity to investigate DAergic activity in extrastriatal areas may yield low signal-to-noise ratio, thus limiting quantification(11, 24). Although [^{18}F]fallypride has been used to quantify DA release in cortical regions due to its high affinity and specificity, the effects of amphetamine on extrastriatal DA release quantified using [^{18}F]fallypride have not been uniformly consistent(25-29). While this has been attributed to the radioligand's inherent signal to noise ratio(26, 28), within-subject variation introduced by two-day scanning protocols, with control and experimental scan on separate days, may also constitute a source of measurement error, particularly in the context of subtle changes in neurotransmitter activity. In order to minimize within-subject variation, we utilized a validated single infusion [^{18}F]fallypride paradigm which circumvents subtraction and yields a model fit approach(3, 30, 31).

In the current study, medication-free NAPD and HV underwent a well-validated experimental psychosocial stress paradigm, the Montreal Imaging Stress Task (MIST)(2). All participants completed a MIST control and stress condition in a single [^{18}F]fallypride session. Subjective stress responses, psychotic symptoms and plasma cortisol levels were assessed throughout each condition. Conform previous work, we first investigated stress-induced [^{18}F]fallypride displacement and the spatial extent of stress-induced [^{18}F]fallypride displacement in mPFC(5, 6), after which we explored other extrastriatal regions. It was expected that both outcome parameters of DA signaling would be positively associated with the subjective stress response in HV. Consistent with the notion of DA-mediated hypofrontality, we expected that NAPD would show less stress-induced extrastriatal [^{18}F]fallypride displacement and a decrease in the spatial extent of stress-induced extrastriatal [^{18}F]fallypride displacement, compared to HV.

METHODS AND MATERIALS

Sample

The sample consisted of 12 HV (unrelated to Lataster et al.(5)) and 12 NAPD matched on age, gender and education (table 1). All NAPD were diagnosed with a non-affective psychotic disorder (supplemental table 1). Four included NAPD were antipsychotics naïve. Except for 1 NAPD, the remaining group was treated with antipsychotics for less than 2 years. At time of scanning, NAPD were off antipsychotics for at least 1 year

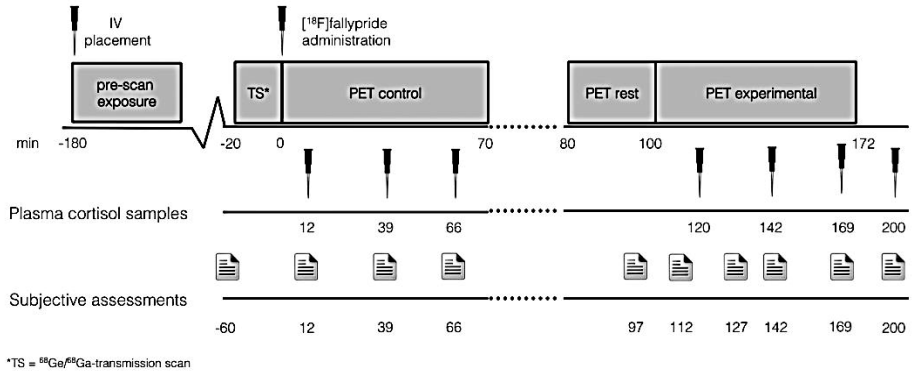
(table 1), were not exposed to mood stabilizers, were off antidepressants (total n=5) for longer than 1 year and did not take benzodiazepines on the day of the scan (supplemental material 1). NAPD showed relatively low acute psychotic symptom scores (table 1), but did not meet the criteria for remission according to the Positive and Negative Syndrome Scale (PANSS) criteria (less than a score of 3 on all relevant items according to van Os et al.(32)). HV were matched to NAPD with a past of minimal illicit drug use (table 1).

Participants were recruited through regional and national media and, additionally, NAPD were recruited through local mental health services. The RWTH Aachen University ethics committee approved the study. PET approval was granted by the national authority for radiation protection in humans in Germany (Bundesamt für Strahlenschutz, BfS). Written informed consent was obtained before participation. Inclusion criteria independent of group: I) age 18-60 II) able to provide informed consent. Exclusion criteria independent of group: I) current/past use of illicit drugs according to the Composite International Diagnostic Interview (CIDI) (WHO, 1990) (lifetime: >15 times cannabis, >5 times other drugs; illicit drug use in the past year), II) foreign bodies precluding a magnetic resonance imaging (MRI) scan, III) neurological disease, IV) pregnancy. NAPD-specific inclusion criterion: diagnosis of non-affective psychotic disorder according to the diagnostic and statistical manual of mental disorders (DSM-IV) criteria. HV-specific exclusion criteria: lifetime history of psychiatric illness according to DSM-IV criteria and lifetime neuroleptic use. On the day of scanning, a urine screening was performed to exclude current drug use and pregnancy.

Psychosocial stress paradigm

Psychosocial stress was induced using the MIST(2). The MIST is a mental arithmetic task with an evaluative psychosocial component and has been prescribed in detail before(1, 2, 5, 33). Psychosocial feedback during the MIST was scripted. All participants were exposed to identical feedback by an investigator who was previously unknown to them. Time and difficulty were automatically adjusted during the experimental condition using a computer algorithm preventing users from exceeding 60 to 70% correct answers. The MIST training version was practiced for 15 minutes at least 2 hours before scan. Participants completed 10 6-minute blocks of MIST control and experimental version. Control and experimental sessions were separated by a break (figure 1).

Figure 1: Graphical overview of the single infusion design



Following the transmission scan, the radioligand was injected after which participants always performed the control block of the MIST for 70 min. After a 10-minute break, participants were repositioned using the scanner coordinate system and reference skin marks. At 100 minutes post-injection, participants performed the MIST experimental condition for 70 minutes. Plasma cortisol samples were collected in intervals ranging from 22 to 54 minutes

Behavioral and physiological assessments

PANSS positive, negative and general symptoms[34] were assessed by a trained researcher before the scan. Subjective stress and psychotic symptoms were briefly assessed pre scan (n=1), during each PET part (n=8) and post scan (n=1) (figure 1). Subjective stress responses were assessed using 7-point Likert Scale items: "I feel relaxed" (reversed), "I feel judged" and "I do not live up to expectations", based on previous work (α =.69)[5, 33]. Psychotic symptoms (positive only) during the scan were assessed using the following items: "I hear voices", "I see things" and "I feel suspicious" (α =.7). Plasma cortisol samples were also collected throughout each PET part (n=6) and post scan (n=1) (figure 1). Plasma cortisol levels were determined using a radio immunoassay[35].

Image acquisition and analyses

MRI T1-weighted MRI scans were acquired on a 1.5T Philips (Philips Medical Systems. Herrsching, Germany) machine with TE=4.59ms, TR=30ms, matrix dimensions= 256x256, slice thickness=2mm, slice number=176. During the data acquisition phase this scanner was replaced by a Siemens 3T scanner (Siemens Healthcare. Munich, Germany). Remaining scans (37,5%) were collected using the Magnetization Prepared Rapid Acquisition Gradient-Echo (MP-RAGE) sequence, with TE=2.52ms, TR=1900ms,

matrix dimensions= 256x256, slice thickness=1mm, slice number=176. A similar proportion of HV (5/12) and NAPD (4/12) MRI scans were obtained on the second machine.

Radioligand preparation The radiosynthesis of [^{18}F]fallypride was a high-yield modification of the synthesis method for [^{18}F]desmethoxyfallypride, described in detail previously[36, 37].

PET acquisition All PET measurements were performed in supine position in a quiet environment. Head position was fixed using a vacuum plastic mould, in order to limit head movement[38]. Scans were performed in three-dimensional mode on a Siemens ECAT EXACT HR+ scanner (Siemens-CTY, Knoxville, TN, USA). Sixty-three slices of 2.425mm slice thickness (pixel size=2mm x 2mm) were reconstructed per time frame by filtered back projection (Hamm filter) after Fourier rebinning into two-dimensional sinograms. Data sets were corrected for random coincidences, scatter radiation and attenuation (10min ^{68}Ge/^{68}Ga-transmission scan). The image matrix was 128x128. PET data were smoothed (4mm FWHM), realigned, co-registered (transformation matrix based on first 10 realigned frames) and normalized to individual T1 MRI (PMOD v3.1 (PMOD Technologies Ltd., Zurich, Switzerland)) and normalized (SPM 8, Wellcome Trust, UK). For every participant, an attenuation-corrected average image of the first 15 minutes was created. These frames were chosen because of their minimal amount of movement and subsequent high signal-to-noise ratio[39]. The remaining frames were realigned to the 15-minute mean image using squared difference sum (dissimilarity function) and trilinear interpolation as rigid matching settings in PMOD v3.1 and inspected frame-by-frame. To quantify the discrepancy between the mean frame and remaining frames, individual datasets X, Y, Z and pitch, roll, yaw parameters were exported from SPM 8 (realign option with trilinear interpolation). HV and NAPD did not differ in movement parameters (data upon request) and total sample movement parameters were low (X, Y, Z movement all <5mm and pitch, roll, yaw all <5°).

Data were collected in two segments, a control and experimental part, in a single session with single bolus administration[3, 33]. The PET acquisition protocol is visualized in figure 1. Dynamic frames were collected every 60s for the first six minutes, after which they were collected every 120s for the remainder of the emission scan, in accordance with previous work[3]. Break frames typically consisted of frame 39 to 42 and were discarded before preprocessing.

PET analysis Time-activity curves (TAC) were obtained for the cerebellum (reference region) and temporal and frontal regions. Two masks were created: one containing cerebellum only and another containing all regions (results section). Regions were based on Brodmann definitions, identical to previous work[5, 33]. Using the Automated Anatomical Labeling mask provided by PMOD v3.1, hippocampus and amygdala were located for all participants. Using the PMOD v3.1 crop and tailor functions, hippocampus and amygdala were drawn and inspected slice-by-slice to ensure mask cov-

erage. All masks were custom-tailored to the individual's MRI, transferred to co-registered PET data in PMOD v3.1 (PMOD Technologies Ltd., Zurich, Switzerland) and visually inspected for fit by two independent individuals. Given that striatal and extrastriatal regions differ in time to reach pseudo equilibrium, stress-induced [^{18}F]fallypride displacement in striatal regions was not investigated; these values could not be reliably investigated with the current design, which was optimized to detect extrastriatal DA signaling[31].

PET data were analyzed using a modified simplified reference tissue model (SRTM)[40], in accordance with previous work[3, 30, 31, 33, 41-44]. Stress-induced [^{18}F]fallypride displacement, reflecting DA release, was quantified using TAC plots and receptor kinetic parameters. The statistically significant change in radioligand displacement was calculated for every region of interest (ROI) as the Z-value of γ (γ/std(γ))[33, 41]. Here, γ is considered an additional time-varying parameter in the SRTM estimating the amplitude of ligand displacement at start of the experimental condition in a single scan session (based on the assumption that changes in competition between DA release and radioligand competition are reflected in the estimation of γ[31]). Given that this design does not assume a physiological steady state, it is suitable to investigate short (phasic), time-varying changes in DA concentrations. The Z-value of γ as a proxy of stimulus-induced changes in DA release is highly correlated with binding potential relative to non-displaceable radioligand (BP_{ND})[33, 41] and has been validated using [^{18}F]fallypride[43].

γ was calculated over an exponential decay function h(t)=exp[−τ(t−T), where t=measurement time, T=time of experimental condition initiation and τ controls the rate at which activation effects die away (dissipation rate, set to τ=0.03 min^{-1} [3, 31, 43]), yielding a γ variate estimation interval peaking at 11 minutes after experimental condition onset, with the peak dissipating to 10% in 69 minutes.

Because previous work has demonstrated that psychological paradigms do not only affect the intensity (amount) of DA release, but also the area affected[3, 33, 45], the spatial extent of [^{18}F]fallypride displacement was calculated as the percentage of voxels in an ROI showing significant radioligand displacement (quantified as γ) after correction (p(/number of total voxels)). This approach requires that voxel T-values in a given region of interest are homogenously distributed for groups of interest (HV, NAPD); this assumption was tested by calculating the decrease in number of active voxels (i.e. significant γ values) when increasing the T-value by 1 (tested for multiple T values) in all ROIs and comparing this between groups (data upon request). High correlations (up to r=.87) between ROI ligand displacement and the spatial extent of ligand displacement (in voxels) were observed, suggesting that the area of DA release increases with DA release.

Analyses

Similar to previously published work investigating stress-induced [^{18}F]fallypride displacement[6] and the spatial extent stress-induced [^{18}F]fallypride displacement[3], the total sample consisted of 12 matched HV and NAPD. A-priori power analyses indicated a power of .82 to detect a group difference which is comparable to previous work using [^{18}F]fallypride[3].

Multilevel regression models with subject as the within level were applied to investigate increases in subjective stress and (positive) psychotic symptoms from control to experimental condition. Difference scores (stress-control condition) for subjective stress/symptoms were calculated for follow-up analyses. The area under the curve (AUC)[46] was calculated for plasma cortisol levels (nmol/l). AUC or nmol/l cortisol difference values were used for all cortisol analyses.

Two measures of stress-induced radioligand displacement were used: [^{18}F]fallypride displacement in an ROI and the spatial extent of [^{18}F]fallypride displacement (see PET analysis section). Regions with mean BP_{ND}<.5 in HV were not taken into account to prevent a low signal-to-noise ratio.

To replicate previous findings, we first investigated stress-induced mPFC [^{18}F]fallypride displacement and the spatial extent of stress-induced mPFC [^{18}F]fallypride displacement in HV. This was followed by an attempt to discover additional extrastriatal regions involved in stress processing in HV (table 2 for all identified regions). For these purposes, t-tests (spatial extent/radioligand displacement>0) were performed. The same procedure was repeated for NAPD; no additional regions were identified in NAPD. Next, group differences (HV vs. NAPD) in stress-induced radioligand displacement and its spatial extent were investigated in regions showing significant stress-induced radioligand displacement (using ANOVA).

Follow-up analyses were performed using stress-induced increased in subjective stress/psychotic symptoms, symptom scores on PANSS subscales (positive, negative, general)[34] and the amount of years off antipsychotics (day of scan – last day of antipsychotics use/365) as outcome variables. α was set to the conventional threshold of p=.05. Given the matched nature of the samples, covariates were not included in group comparisons. When analyzing single groups, age and gender were entered as nuisance covariates.

RESULTS

Demographics, behavioral and physiological assessments

Groups did not differ on demographic variables (table 1; all n.s.). Recreational illicit drug use ceased long before the scan and no included participants reported current

drug use (years since last use (M = 17.83, SD =7.52). Antipsychotics naïve NAPD (n=4) and antipsychotics free (currently non-medicated > 1 year) participants did not differ in their PANSS score on the positive subscale (t(1,23)=.25, p=.81). Subjective stress during the scan increased from control to experimental condition (b=.63 z(188)=6.07, p=<.0001), regardless of group (b=-.24, z(1,188)=-1.14, p=.26). NAPD increased in positive psychotic symptoms from control to stress condition (b=.21 z(95)=2.79, p=.005). Subjective stress in the whole sample (b=-1.24, z(116)=-7.93, p=<.001) and positive psychotic symptoms in NAPD (b=-.26, z(58)=-2.21, p=.03) significantly decreased following a debriefing session 15 minutes after the scan finished. Cortisol (nmol/l) decreased as a function of time in HV (b=-.34, t(64)=-2.87, p=004), but not in NAPD (b=-.02, z(66)=-.11, p=.91).

There were no differences in AUC cortisol between conditions (t(18)=1.65, p=.12), nor were there group differences (b=474.42, t(1,9)=.21, p=.83) in AUC cortisol difference scores or an association with subjective stress (b=671.38, t(18)=.43, p=.67).

Table 1: Sample demographics

	HV	NAPD	Statistics (p-value, test statistic)
Gender			(1, 0[A])
Male	8	8	
Female	4	4	
Age	48.08 (9.94)	44.67 (11.24)	(.44, -.79[B])
Education[1]	5.83 (1.4)	5.33 (1.44)	(.4, .86[A])
Smoking			(.38, .54[A])
Nonsmoker	11	10	
Smoker	1	2	
Cannabis lifetime[2]	.23 (.83)	.67 (1.23)	(.31, 1.04)
Other drugs lifetime[2,3]	0 (0)	.01 (.04)	(.31, 1.04)
Injected radioligand (MBq)	189.83 (8.2)	187.92 (10.86)	(.4, -.85[B])
Specific activity (GBq)	2611.42 (872.96)	2146.25 (1198.6)	(98, -.03[B])
Current symptoms[4]	-	11.83 (3.93)	-
Years off AP	-	7.09 (4.96)	-
Cumulative haloperidol equivalents[5]	-	4303.07 (12280.64)	-

[1]highest finished education, scored on a scale ranging from 1(primary school) to 8 (Master's degree)
[2]lifetime use scored on a scale ranging from 1(1-5 times) to 8 (>100 times)
[3]stimulants, sedatives, opiates, cocaine, psychedelics, XTC, MDMA, PCP and inhalants subscales
[4]positive subscale of the Positive and Negative Syndrome Subscale (PANSS)
[5]cumulative haloperidol equivalents were calculated by converting the weekly antipsychotics dose to haloperidol equivalents and multiplying it by the number of weeks the antipsychotics were taken.
[A]=Chi2, [B]=T-test

Stress-induced [^{18}F]fallypride displacement: main effects and group differences

Average HV BP_{ND} calculated over the whole paradigm using the SRTM[40] in the mPFC (M=.51, SD=.2), temporal cortex (TC) (M=.63, SD=.16), hippocampus (M=1.56, SD=.88), parahippocampal gyrus (M=.66, SD=.18) and amygdala (M=4.13, SD=1.56) was higher than .5 These regions were therefore included in the mask. No additional regions with BP>.5 were identified in NAPD.

In the mPFC and TC, a significant stress-induced increase in radioligand displacement and the spatial extent of radioligand displacement could be observed in HV and NAPD separately ($p<.05$), but not in the hippocampus, parahippocampal gyrus or amygdala ($p>.05$). No group differences in stress-induced radioligand displacement were observed in a-priori selected region of interest the mPFC (table 2), nor when looking at the dorso-mPFC ($b=-.05$, $t(1,23)=-.12$, $p=.91$) or ventro-mPFC ($b=-.09$, $t(1,23)=-.23$, $p=.82$) subregions separately. Moreover, no group differences in stress-induced radioligand displacement were observed in the TC (table2).

Similarly, no group differences were observed in the spatial extent of stress-induced radioligand displacement in the mPFC (table 2), dorso-mPFC ($b=-3.11$, $t(1,23)=-.55$, $p=.59$), ventro-mPFC ($b=-6.86$, $t(1,23)=-1.3$, $p=.21$) or TC (table 2) (figure 2, 3).

Table 2: Extrastriatal stress-induced [^{18}F]fallypride displacement in HV and NAPD

Region	HV Stress-induced [^{18}F]fallypride displacement Mean Z(γ)^{A} (SD)	NAPD Stress-induced [^{18}F]fallypride displacement Mean Z(γ)^{A} (SD)	P-value group diff. Z(γ)	T-value group diff. Z(γ)	HV Spatial extent of stress-induced [^{18}F]fallypride displacement Mean n%^{B} (SD)	NAPD Spatial extent of stress-induced [^{18}F]fallypride displacement Mean n%^{B} (SD)	P-value group diff. spatial extent	T-value group diff. spatial extent
Frontal lobe								
MPFC*	2.68 (3.99)	1.59 (2.98)	.46	-.76	24.92 (12.16)	19.12 (10.67)	.23	-1.24
Temporal lobe								
Temporal CTX*†^{C}	12.88 (4.02)	13.79 (3.02)	.54	3.21	8.96 (6.49)	7.34 (7.05)	.27	-1.14
Hippocampus†	-.42 (1.08)	-.39 (.74)	.95	.06	<1%	1.8 (3.1)	.3	1.06
Parahippocampal gyrus†	-.59 (82)	-.72 (.47)	.65	-.45	1.34 (1.83)	1.08 (1.18)	.68	-.42
Amygdala†	-.17 (.13)	-.48 (.83)	.47	-.74	2.07 (6.38)	<1%	.43	-.81

*significant stress-induced increase in tracer displacement and spatial extent of tracer displacement in HV and NAPD (p<.05)

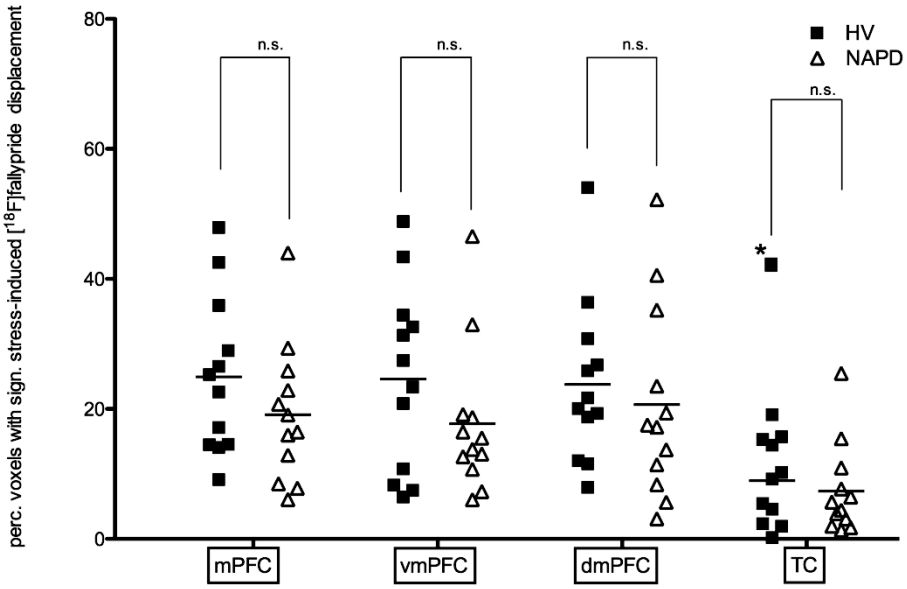
^{A}=stress-induced [^{18}F]fallypride displacement (Z(γ))

^{B}=% of total voxels in ROI showing significant stress-induced [^{18}F]fallypride displacement (z-value of γ)

^{C}=temporal cortex (inferior and superior temporal gyri)

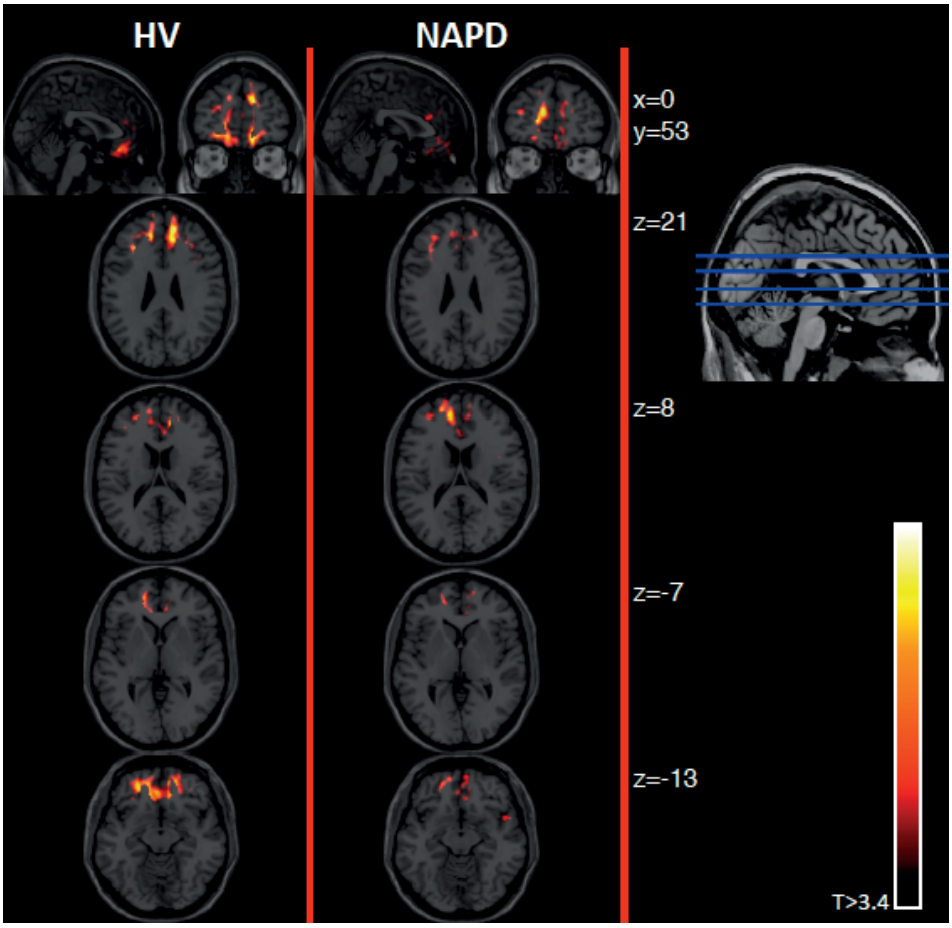
†=one outlier removed with Cook's distance > 4/12

Figure 2: Group averages for the spatial extent of stress-induced [^{18}F]fallypride displacement



NAPD did not significantly differ from HV in the spatial extent of stress-induced [^{18}F]fallypride displacement in any (sub)region. Ventro-mPFC and dorso-mPFC are mPFC subregions. *=outlier (Cook's distance > 4/n), removed from mean. N.s. = not significant at p=.05.

Figure 3: Parametric maps showing stress-induced [^{18}F]fallypride displacement in mPFC



Graphical representation showing stress-induced [^{18}F]fallypride in HV and NAPD in coronal (top row, left images), sagittal (top row, right images) and axial view (columns). Coronal image and Montreal Neurological Insititute (MNI) Z coordinates on the right depict the axial slice position. Starting position (top) was x=0, y=53, z=21 (MNI). Mean t-maps per group show the stress-induced [^{18}F]fallypride displacement throughout the mPFC. Individual t-maps were generated using displacement parameter γ (t=γ/sd(γ) and were averaged across all participants per group. Images are thresholded at 3.4 for visualization purposes.

Stress-induced [^{18}F]fallypride displacement: follow-up analyses

In the whole sample, stress-induced radioligand displacement in mPFC ($F(23)=.11$, $p=.74$) or TC ($F(23)=.88$, $p=.36$) was not associated with subjective stress. The association between the spatial extent of stress-induced mPFC radioligand displacement and subjective stress in the whole sample did not reach significance ($F(23)=1.71$, $p=.2$). When looking at mPFC subregions, the association between subjective stress and the spatial extent of stress-induced radioligand displacement in ventro-mPFC ($F(23)=2.48$,

p=.09) and dorso-mPFC (F(23)=.15, p=.87) was not significant (figure 4). However, further investigation revealed a significant positive association between subjective stress and the spatial extent of stress-induced radioligand displacement in right ventro-mPFC (F(23)=4, p=.03) (figure 4), but not left ventro-mPFC (F(23).83, p=.45). Subjective stress was not associated with the spatial extent of stress-induced radioligand displacement in TC (F(23)=.63, p=.54) (figure 4).

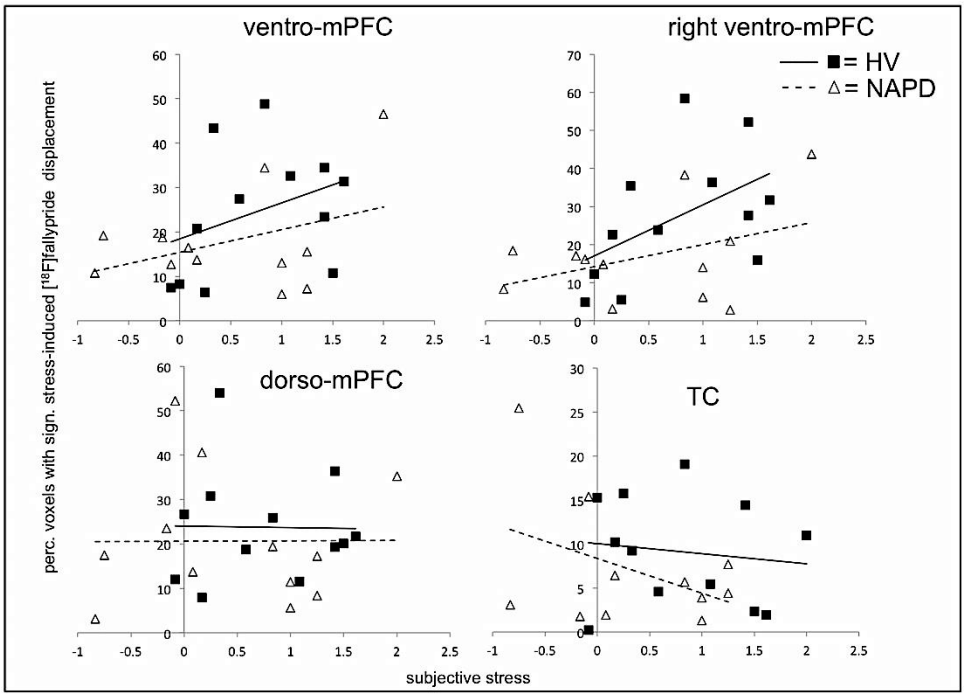
The spatial extent of stress-induced radioligand displacement (b=1.13, t(7)=7.75, p=.001), but not stress-induced radioligand displacement (b=-.22, t(7)=-2, p=.12), in ventro-mPFC was positively associated with duration of antipsychotics free period.

Psychotic symptoms during the scan in NAPD were not associated with stress-induced radioligand displacement in mPFC (b=.1.25, t(11)=-.13, p=.9) or TC (b=-1.42, t(11)=-.67, p=.53), or the spatial extent of stress-induced radioligand displacement in mPFC (b=-3.68, t(11)=.51, p=.62) or TC (b=-3.24, t(11)=-.63, p=.55). PANSS positive, negative or general symptoms in NAPD were also not associated with stress-induced radioligand displacement or the spatial extent of stress-induced radioligand displacement in mPFC or TC (table 3).

Adding years off antipsychotics as a covariate did not change the results. Moreover, antipsychotics naïve NAPD and antipsychotics free participants did not differ in stress-induced radioligand displacement or the spatial extent of stress-induced radioligand displacement in any of the identified regions (data not shown).

Finally, cumulative haloperidol equivalents (antipsychotics in the past) were not associated with stress-induced radioligand displacement in mPFC (b=<.01, t(11)=-.47, p=.65) or TC (b=<.01, t(11)=1.05, p=.32), or the spatial extent of stress-induced tracer displacement in mPFC (b=<-.01), t(11)=-.61, p=.55) or TC (b=<-.01, t(11)=-.37, p=72).

Figure 4: Association between the spatial extent of stress-induced [^{18}]fallypride displacement and subjective stress in the whole sample



Subjective stress and the spatial extent of stress-induced radioligand displacement were associated in ventro-mPFC (trend, p=.06) and, more specifically, right ventro-mPFC (p=.02) in the whole sample, but not in dorso-mPFC (p=.93) and TC (p=.33). For visualization purposes, HV and NAPD were depicted separately.

Table 3: Associations between stress-induced [^{18}F]fallypride displacement and psychotic symptoms on the Positive and Negative Syndrome Subscale (PANSS) in NAPD

	Association between stress-induced [^{18}F]fallypride displacement (Z(γ)) and PANSS symptoms				Association between spatial extent of stress-induced [^{18}F]fallypride displacement (% voxels) and PANSS symptoms			
	Coefficient	95% CI	T-value	P-value	Coefficient	95% CI	T-value	P-value
Positive subscale								
mPFC	.16	-.36 to .69	.72	.5	.88	-1.09 to 2.84	1.03	.33
Temporal CTX	-.05	-.68 to .56	-.22	.84	.9	-.42 to 2.21	1.57	.16
Negative subscale								
mPFC	.29	-.95 to 1.53	.54	.61	-.45	-5.26 to 4.37	-.21	.84
Temporal CTX	-.7	-2.02 to .62	-1.22	.26	.49	-2.97 to 3.94	.33	.75
General subscale								
mPFC	.05	-.58 to .52	-.12	.91	.26	-1.83 to 2.34	.28	.78
Temporal CTX	-.16	-.77 to .45	-.6	.57	.48	-.98 to 1.94	.76	.47

DISCUSSION

Using [^{18}F]fallypride PET, the effect of psychosocial stress on extrastriatal DA signaling was investigated in HV and NAPD. In accordance with previous work, extrastriatal DA release(6) and the spatial extent (area/size of DA release in voxels) of DA release(3, 31) served as primary outcome measures of stress-related DA signaling. We showed that psychosocial stress increases extrastriatal DA signaling in HV: both DA release and the spatial extent of DA release increased in mPFC and TC. Moreover, we did not find evidence for altered stress-induced extrastriatal DA signaling in NAPD. This is based on the observations that I) psychological stress increased both outcome measures of stress-related DA signaling to a similar extent in HV and NAPD, II) subjective stress and the spatial extent of stress-induced DA release were similarly associated in HV and NAPD and III) both outcome measures of stress-related DA signaling were not associated with positive, negative or general symptom scales of the PANSS in NAPD(34).

BP_{ND} values in frontal and temporal areas were in ranges comparable to previous studies(6, 28) although inter-individual variability was observed in the hippocampus and amygdala, which may be the result of the inherent small size of these structures. The observation that stress increased mPFC DA signaling in HV confirm previous data(5, 6). Additionally, increases in DA signaling in TC were observed. Although stress-induced TC DA signaling in humans has not been reported before, it is consistent with functional magnetic resonance imaging studies using the MIST(47, 48), suggesting that these effects might be, in part, DAergic.

Contrary to expectations, differences in stress-induced frontal and temporal DA signaling between HV and NAPD were not observed. In combination with the absence of a correlation between measures of stress-induced DA signaling and psychotic symptoms (during scan or assessed with PANSS), these results could suggest that stress-related extrastriatal DA signaling is unaffected in NAPD: increased (hyper) stress-induced striatal DA release observed in the context of psychotic disorder, reported by others(1, 2, 4), may not necessarily co-occur with changes in extrastriatal DAergic activity. Here, we offer four explanations.

Firstly, these results seemingly contrast with the hypothesis of DA-mediated hypofrontality in psychosis(18, 19). However, the concept of hypofrontality is often assessed indirectly (e.g. cerebral blood flow) and in the context of cognitive performance(18, 49, 50), not stress. Little *in vivo* evidence exists for $D_{2/3}$-mediated hypofrontality in psychotic disorder(10) and a positive association between amphetamine-induced PFC DA release measured with [^{18}F]fallpyride and schizotypal personality traits(29) may even suggest increased cortical DA transmission in psychotic disorder. Although inconsistent(51-53), DAergic hypofrontality in psychotic disorder has been observed with D_1 molecular imaging ligands. Moreover, experimental animal work suggests an important role for PFC D_1 receptors in the stress response(54) and a D_1, but not D_2, agonist can restore stress-related DAergic PFC-striatum interactions (55).

Altogether, this could indicate that, while DA transmission at $D_{2/3}$ during stress may be unaltered in psychotic disorder, activity at the D_1 may be abnormal.

Secondly, the absence of differences between HV and NAPD could be explained by the relatively low amount of acute psychotic symptoms (PANSS score; table 1). However, it is generally acknowledged that increased stress-sensitivity is present in those at risk for psychotic disorder(56), non-acute psychotic disorder(57) and even remitted psychotic disorder(58). In addition, stress-induced increases in psychotic symptoms during the scan confirmed abnormal stress-sensitivity in our sample of NAPD. We recently reported a negative correlation between the spatial extent of mPFC DA release and subjective stress/subclinical psychotic symptoms in healthy first-degree relatives of individuals with psychotic disorder(3). This could suggest functional cortical DAergic alterations in the stress response in some, but not all, individuals across the psychosis continuum. One way to investigate if stress-related PFC DA signaling is dependent on illness phase is the addition of a group of acutely psychotic NAPD.

A third explanation may be that the use of [^{18}F]fallypride has contributed to the absence of group differences. Amphetamine-induced cortical DA release quantified with fallypride has yielded inconsistent results(25, 27, 28). However, three separate studies using the MIST(3, 6) (including the current one), as well as a study investigating response inhibition(59), have reported cortical DAergic activity measured with fallypride. The discrepancy between stimulant- and task-based studies may stem from the different mechanisms by which DA can be released: whereas psychological tasks elicit DA release through increased cell firing(60), stimulants increase extracellular DA release through DA and noradrenaline transporter blockade(61) as well as decreasing cell firing(62). A replication study with higher affinity radioligands such as FLB 457(26, 63) could be useful to assess the suitability of fallypride to detect task-induced cortical DA release, as has been done recently for stimulants(26).

A final explanation could be that the sample displayed abnormalities in cortical neurotransmission not related to the DA system. This assumption is based on the observation that cognitive and negative symptoms in NAPD were not associated with stress-related DA signaling. One potential candidate neurotransmitter system could be glutamate. Glutamate transmission in the cortex plays an essential role in stress processing(64), and cognitive and negative symptoms of schizophrenia have been associated with altered frontal glutamate activity(65), but not always consistently so(66). Thus, alterations in cortical glutamate transmission could potentially account for negative and cognitive symptoms in the sample of NAPD whilst also explaining their increased stress-sensitivity to the task.

While there may be multiple explanations for the absence of differences between NAPD and HV, stress-induced mPFC DA release(6) and the spatial extent of mPFC DA release(3) are associated with physiological and behavioral parameters. This suggests that PFC DAergic processing plays a functional role in the stress response, which is potentially unaltered in NAPD. This role is once again confirmed by the correlation

between the subjective stress response and spatial extent of ventro-mPFC DA release in the current study. However, an association between subjective stress and ventro-mPFC DA release was not observed. Although high correlations were observed between the spatial extent of DA release and DA release, this may indicate that increases in subjective stress are associated with a greater area of DA release without altering the amount of DA released. This could be interpreted as a compensatory processing mechanism, where increased resources are necessary to obtain the same result.

The spatial extent of ventro-mPFC DA release in response to stress increased as NAPD were longer off antipsychotics. Two possible explanations exist for this association. Firstly, as NAPD are longer off antipsychotics, their DAergic stress response may progressively approximate that of HV. This is in line with an association between D_1 receptor density and drug free interval(67) and could suggest that DA receptor density may normalize following prolonged exposure to antipsychotics. The association between time off antipsychotics and the spatial extent of stress-related DA release may reflect gradual homeostatic down-regulation of PFC $D_{2/3}$ receptors, previously up-regulated through extended antipsychotics blockade, although such up-regulations are dependent on mode of antipsychotics administration(68, 69).

An alternative explanation may be that as acute psychotic symptoms decrease, DAergic abnormalities normalize. This is in line with work showing that striatal DA function of remitted schizophrenia patients(70) and antipsychotics-treated schizophrenia patients(71) is similar to HV. However, this explanation goes against alterations in stress-sensitivity that persist beyond acute psychotic disorder(58) and the observation that the MIST increased psychotic symptoms in NAPD. Here, again, an acutely psychotic group of NAPD could be of added value.

STRENGTHS AND LIMITATIONS

The current findings need to be interpreted in light of strengths, limitations and sample size.

Strengths of the study include minimal past drug use in the sample, thereby excluding substance-induced NAPD and associated confounds in the DA system. Given that, in particular, cannabis use is associated with psychotic symptoms(72) and DA function(73-75), this may have increased our sensitivity to investigate stress-related DA function. Moreover, the single infusion paradigm limited within-subjection variation, further decreasing measurement error. Finally, the direction and location of task effects in HV were similar to a previous study using an identical design, suggest a degree of stability(5).

Some limitations of the study need to be addressed. A general limitation is that the single infusion protocol with fallypride employed in the current study has not been associated with measures directly related to DA activity, hence use of the term “DA

signaling". Moreover, striatal DA signaling could not be reliably investigated; actual and simulated data(31) indicate that the current design would produce unreliable estimates for the striatum, given the slow time course of radioligand binding. Future [^{18}F]fallypride studies could increase scan duration or, in the case of a single infusion paradigm, prolong the control condition to investigate striatal and extrastriatal DA signaling simultaneously.

Because of model assumptions and in order to limit stress exposure to the scanning period, the task order was fixed to control-experimental, similar to previous work(5, 14). Although this may have introduced order effects, a recent [^{18}F]fallypride PET study demonstrated stress-induced DA release independent of the order of conditions(6). This makes it unlikely that order effects had a major effect on our outcome measures.

In addition, benzamide binding is affected by cerebral blood flow(76) However, in response to behavioral challenges(43) and in low-binding areas(77), regional cerebral blood flow effects are rather small and are not expected to explain the presented results. Other studies with a single infusion paradigm have discussed this issue in greater detail(5, 30, 33, 45).

In the absence of a task-induced effect on plasma cortisol levels, our results could reflect socially desirable behavior or increased effort in the stress condition. The association between subjective stress and the spatial extent of ventro-mPFC DA release does however suggest an effect of the stressor. This is also confirmed from by data from one HV who was scanned in a control-control sequence (data upon request); changes in subjective stress or [^{18}F]fallypride displacement were not observed.

Rather, the absence of cortisol effects may be related to time of day; a significant association between sampling time and cortisol nmol/l in HV was observed. Both the current study as well as another recent study who failed to find an effect of the MIST on cortisol levels(6) collected PET data in the afternoon. In contrast, in a previous study we did find an effect of the MIST on cortisol levels, but PET data were collected around noon. Future studies may therefore want to include physiological parameters that are less sensitive to time of day than cortisol.

Another observation was that MIST effects on the spatial extent of stress-induced ventro-mPFC DA release were smaller than previous work using an identical acquisition protocol (~25% here vs. ~50%(3)). This may be related to different versions of the task; whereas the current study used an auto-adjust version (set to 70% correct responses), a manually calibrated task (aiming at 90% correct response) was used previously. Task differences may have affected the perceived stressfulness of the paradigm and, correspondingly, DAergic processing. Moreover, image preprocessing software, scanner type and head fixation procedures may further explain these between-study differences.

Some limitations related to the sample also need to be addressed. Although NAPD were off antipsychotics for longer than 1 year, past antipsychotic use may have affected DA receptor density and thus masked subtle illness-related effects on stress-

induced DA signaling. While this is a limitation we acknowledge, repeating the analyses with time off antipsychotics as a covariate did not affect the results described in this manuscript. A sample of neuroleptic naïve participants could be valuable in detecting alterations in the extrastriatal DAergic stress response, if any, associated with NAPD. In addition, the NAPD sample included five individuals with brief psychotic disorder as their main diagnosis (supplemental table 1); low-grade residual symptoms in these individuals may have limited the power to detect associations between stress-related DA signaling and psychotic symptoms. Finally, post-hoc power calculations indicated that group differences with effect sizes (Cohen's *d*) up until .5 may have been overlooked. In order to detect small to moderate group differences, replication with larger sample sizes is essential.

CONCLUSIONS

Preclinical(16, 17) and human(3) studies have previously shown that stress affects DAergic activity in frontal cortical areas. Although hypofrontality has been proposed to be an important feature of psychotic disorder(18, 19), the preliminary evidence presented here does not provide evidence for altered extrastriatal DA signaling in the context of stress in NAPD. While we have demonstrated that frontal DA signaling is functionally relevant in the stress response, it is not clear how this related to the putative link between stress and psychotic disorder. Follow-up studies in acutely psychotic and neuroleptic-naïve NAPD could provide new insights into the role of stress-related extrastriatal DAergic processing in NAPD.

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Chapter 6

Early-life stress affects stress-related prefrontal dopamine activity in healthy adults, but not in individuals with psychotic disorder

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ABSTRACT

Early life stress may have a lasting impact on the developmental programming of the dopamine (DA) system implicated in psychosis. Early adversity could promote resilience by calibrating the prefrontal stress-regulatory dopaminergic neurotransmission to improve the individual's fit with the predicted stressful environment. Aberrant reactivity to such match between proximal and distal environments may, however, enhance psychosis disease risk. We explored the combined effects of childhood adversity and adult stress by exposing 12 unmedicated individuals with a diagnosis of non-affective psychotic disorder (NAPD) and 12 healthy controls (HC) to psychosocial stress during an [^{18}F]fallypride positron emission tomography. Childhood trauma divided into early (ages 0-11 years) and late (12–18 years) was assessed retrospectively using a questionnaire.

A significant group x childhood trauma interaction on the spatial extent of stress-related [^{18}F]fallypride displacement was observed in the mPFC for early (b=-8.45, t(1,23)=-3.35, p=.004), and late childhood trauma (b=-7.86, t(1,23)=-2.48, p=.023).In healthy individuals, the spatial extent of mPFC DA activity under acute psychosocial stress was positively associated with the severity of early (b=7.23, t(11)=3.06, p=.016), as well as late childhood trauma (b=-7.86, t(1,23)=-2.48, p=.023). Additionally, a trend-level main effect of early childhood trauma on subjective stress response emerged within this group (b=-.7, t(11)=-2, p=.07), where higher early trauma correlated with lower subjective stress response to the task. In the NAPD group, childhood trauma was not associated with the spatial extent of the tracer displacement in mPFC (b=-1.22, t(11)=-0.67), nor was there a main effect of trauma on the subjective perception of stress within this group (b=.004, t(11)=.01, p=.99).

These findings reveal a potential mechanism of neuroadaptation of prefrontal DA transmission to early life stress, and suggest its role in resilience and vulnerability to psychosis.

INTRODUCTION

Adverse early-life experiences such as abuse or parental loss are highly prevalent phenomena in children with reports of up to 60% being exposed to at least one major traumatic event by the time they are 16 years old (1). These statistics become all the more concerning in the light of epidemiological evidence linking traumatic experiences in early life to higher risk for psychosis years later (2, 3). Indeed, a comprehensive meta-analysis of case-control and population-based studies revealed a threefold increase in risk of developing a psychotic disorder among those reporting childhood trauma (4). Moreover, compelling prospective evidence suggests a dose-response relationship between the exposure to early life trauma and incidence of psychotic symptoms (5) and the subsequent need for care (6). Most individuals facing early adversity, however, are resilient to psychosis, and only a small portion descends into psychotic illness (7). Thus, studying the effects of childhood trauma in healthy adults and patients with psychotic disorder can potentially allow to identify some of the neurodevelopmental programming mechanisms fostering resilience to adversity.

Various lines of evidence suggest that psychosis is associated with critical alterations in central stress-regulatory mechanisms affecting neural and endocrine stress systems (8-10), and manifested through maladaptive affective and psychotic reactivity to stress (11, 12). Resilience to psychosis, on the other hand, appears to be promoted by advantageous neuroadaptive changes in the stress-modulatory network, through which early life stress likely exerts a hormetic effect on stress susceptibility later in life (13). The dopamine (DA) system, which has long been the subject of investigation in psychosis (14), has been implicated in these changes (15, 16), making it a prime candidate for explorations into the putative protective versus psychotogenic effects of early life stress.

Preclinical work has revealed the critical DAergic hubs of the stress network: the medial prefrontal cortex (mPFC), nucleus accumbens and striatum (15, 17-19), with recent work proposing a reciprocal relationship between these hubs (20, 21). Several reports suggest that exposure to early life trauma may affect this pathway; rodents exposed to early adversity demonstrate long-lasting stress blunted mPFC DA outflow (22, 23) and increased tonic DA levels in subcortical areas (24). Corroborating evidence in humans has also implied the DA system in stress processing in most of the hubs within the stress network, primarily mediated by $D_{2/3}$ receptors (25-27). Moreover, increased striatal DAergic reactivity to stress has been associated with both childhood adversity (28, 29) and the psychosis continuum (27, 29).

While there is rising support for the role of midbrain DA release in the psychotogenic effects of stress (27, 29), the evidence for the role of the mPFC remains inconclusive. Work of our group recently offered evidence for unaltered stress-related prefrontal DA function in psychosis: the DAergic response to psychological stress in mPFC was similar in healthy controls and patients with a psychotic disorder, and correlated with

subjective experience of stress in the entire sample (25). The effect of early life stress, however, was not taken into account, leaving possible resilience or vulnerbaility mechanisms unidentified.

The current study, therefore, aimed to investigate the effect of childhood adversity on DAergic stress processing in frontal cortical areas in non-medicated patients with non-affective psychotic disorder (NAPD) as well as in healthy volunteers (HV) in order to further elucidate the DAergic contribution to both vulnerability as well as resilience to psychosis. To this end, we used data acquired previously (25) in a single bolus-infusion [^{18}F]fallypride positron emission tomography (PET) during which psychosocial stress was induced using a well-validated Montreal Imaging Stress Task (MIST) (28). Conform previous reports implicating the mPFC in the traumagenic dysfunction and stress modulation (25, 30) the mPFC as a whole, as well as its ventral and dorsal portions, were a priori selected as the regions of interest (ROI). In these regions, we hypothesized a differential effect of childhood trauma on the spatial extent of stress-induced DA release among NAPD and HV. Moreover, differential effects of childhood trauma on the subjective experience of stress during the scan were expected in the two groups.

MATERIALS AND METHODS

Sample characteristics

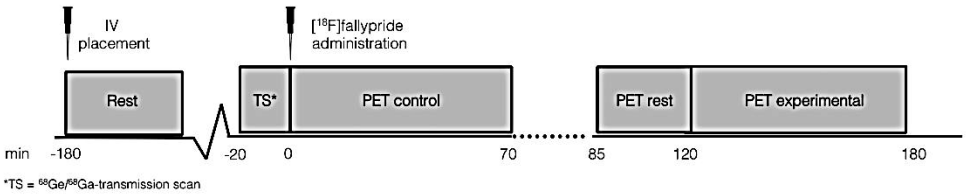
The sample consisted of 12 HV and 12 unmedicated NAPD matched on age, gender and education described in detail previously (Hernaus et al., 2015). All NAPD were currently off antipsychotic medication (AP) for longer than one year, did not currently use mood stabilizers, antidepressants or benzodiazepines. Participants were recruited through regional and national media and, additionally, NAPD were recruited through local mental health services. Inclusion criteria independent of group: I) age 18-60 II) able to provide informed consent. Exclusion criteria independent of group: I) current/past use of illicit drugs according to the Composite International Diagnostic Interview (CIDI; WHO, 1990) (lifetime: >15 times cannabis, >5 times other drugs; illicit drug use in the past year), II) ferromagnetic metal element in or on the body, III) neurological disease, IV) pregnancy. HV-specific exclusion criteria: lifetime history of psychiatric illness according to the diagnostic and statistical manual of mental disorders (DSM) IV criteria and lifetime AP use. NAPD-specific inclusion criterion: diagnosis of non-affective psychosis according to DSM-IV criteria (NAPD were not in remission according to the Positive and Negative Syndrome Scale (PANSS) criteria (31)). On the day of scanning, a urine screening was performed to ascertain current drug use and pregnancy. The RWTH Aachen University ethics committee approved the study. PET approval was additionally granted by the national authority for radiation protection in humans in

Germany (Bundesamt für Strahlenschutz, BfS). Written informed consent was obtained before participation, and participants were treated in accordance with the Declaration of Helsinki.

Psychosocial stress paradigm

Psychosocial stress was induced using the MIST (28), a mental arithmetic task with social evaluative component. During the MIST task, participants were asked to solve arithmetic problems first under a control condition during which no time constraint or feedback were present, and subsequently under the experimental condition where time and difficulty were automatically adjusted to ensure 30-40% error rate. Participants were continuously made aware of their suboptimal performance via a visual performance bar and scripted verbal negative feedback delivered approximately every 12 minutes throughout the experimental condition (6 times in total), during which a confederate researcher reminded the participants that they were performing worse than all previous participants. There were 10 6-minute blocks of MIST control and experimental version (Fig. 1). Dispositional subjective stress and positive symptoms of psychosis were assessed pre scan (n=1), during each PET condition (n=8) and post scan (n=1) (Fig.1) using validated 7-point Likert Scale items. Similar to previous work (11, 25, 30), subjective stress was measured using items with sufficient variability and internal consistency (Cronbach's alpha = .69): "I feel pressured", "I feel judged",and "I'm in control" (recoded). Moreover, positive symptoms of psychosis were assessed using the items: "I hear voices", "I see things" and "I feel suspicious" (Cronbach's α =.7). Factor analyses confirmed that the subjective stress items loaded on a single factor, which was also the case for the psychotic symptoms items. Since the psychosocial stress task was always administered last, it might have been more demanding for patients than for controls, thus influencing their subjective state and brain activity. The perceived difficulty of the current task at hand was thus assessed with the item "this is difficult for me", rated on the same scale.

Figure 1: Schematic representation of the single bolus design



Following the transmission scan, the radioligand was injected after which participants always performed the control block of the MIST for 70 min. After a 10-minute break, participants were repositioned using the

scanner coordinate system and reference skin marks. At 100 minutes post-injection, participants performed the MIST experimental condition for 70 minutes.

Image acquisition and analysys

MRI T1-weighted Magnetic Resonance Imaging (MRI) scans were acquired on a 1.5T Philips (Philips Medical Systems. Herrsching, Germany) machine with TE=4.59ms, TR=30ms, matrix dimensions=256x256, slice thickness=2mm, slice number=176. This scanner was replaced by a Siemens 3T scanner (Siemens Healthcare. Munich, Germany) and all remaining scans (39%) were collected using the Magnetization Prepared Rapid Acquisition Gradient-Echo (MP-RAGE) sequence, with TE=2.52ms, TR=1900ms, matrix dimensions= 256x256, slice thickness=1mm, slice number=176. A similar proportion of scans for HV and NAPD was collected on the second machine (5/12 vs. 4/12).

Tracer preparation The radiosynthesis of [^{18}F]fallypride was a high-yield modification of the synthesis method for [^{18}F]desmethoxyfallypride, described in detail previously (32).

PET acquisition PET measurements were performed in three-dimensional mode on a Siemens ECAT EXACT HR+ scanner (Siemens-CTY, Knoxville, TN, USA). Sixty-three slices of 2.425mm slice thickness (pixel size=2mm x 2mm) were reconstructed per time frame by filtered back projection (Hamm filter) after Fourier rebinning into two-dimensional sinograms. Data sets were corrected for random coincidences, scatter radiation and attenuation (10min ^{68}Ge/^{68}Ga-transmission scan). Image matrix was 128x128. PET data were smoothed (4mm FWHM), realigned (realignment image based on first 15 minutes of the scan), co-registered (transformation matrix based on first 10 realigned frames to individual T1 MRI (PMOD v3.1, PMOD Technologies Ltd., Zurich, Switzerland) and normalized (SPM8, Wellcome Trust, UK). Preprocessing details have been published previousled (25). Data were collected in two segments, a control and experimental part, in a single session with single bolus administration (figure 1) (25, 33).

PET analysis Time-activity curves (TAC) were obtained for the cerebellum (reference region) and temporal and frontal regions. Mask preparation details have been described previously. Briefly, regions were based on Brodmann definitions. Masks were custom-tailored to the individual's MRI, transferred to co-registered PET data in PMOD v3.1 and visually inspected for fit by two independent individuals. PET data were analyzed using a modified simplified reference tissue model (SRTM), in accordance with previous work (33-38). Stress-induced [^{18}F]fallypride displacement was quantified using TAC plots and receptor kinetic parameters. Tracer displacement was calculated for every person on a voxel-wise basis as the standardized value of γ (γ/std(γ)) (38), where γ is considered an additional time-varying parameter in the SRTM estimating the amplitude of ligand displacement at start of the experimental

condition in a single scan session (based on the assumption that changes in competition between DA release and radioligand competition are reflected in the estimation of γ) (34). γ was calculated over an exponential decay function h(t)=exp[−τ(t−T), where t=measurement time, T=time of experimental condition initiation and τ controls the rate at which activation effects die away (dissipation rate set to τ=0.03 min^{-1}; (33, 36, 37). The number of voxels surviving p(/number of total voxels)=.05 reflects the spatial extent of task-induced ligand displacement and was used as primary outcome measure of stress-related DA function. This approach has been validated for [^{18}F]fallypride (36) and has been used to investigate phasic DAergic activity in extrastriatal areas (33, 38).

Childhood trauma assessment

Childhood trauma was measured using Childhood Experience of Care and Abuse (CECA-Q) (39), a validated, retrospective questionnaire to assess childhood trauma in early childhood spanning from 0 to 11 years of age, and late childhood encompassing years 12 through 17. For the purpose of this study, a composite score was created for each time period using 15 dichotomous ('yes'=1 and 'no'=0) items informing about family arrangements, parental loss, physical and sexual abuse, neglect and bullying.

Analyses

Final analyses were performed using STATA 11.2 (StataCorp, 2011). The percentage of voxels in a ROI surviving the Bonferroni-corrected threshold was used as outcome for stress-induced changes in DAergic activity (30, 36, 38). Conform previous results from this sample (25), the mPFC was a priori selected as the primary ROI, with its ventral and dorsal portions (vmPFC and dmPFC, respectively) as two additional ROIs. The percentage of voxels of the left and right regions were summed into a corresponding bilateral ROI and entered into regression analyses as the dependent variable, with the childhood trauma score as the predictor and group (NAPD, HV) as the interaction term. Separate linear regressions were performed for each ROI, and for each trauma timeframe: early and late. Assessments of subjective stress and psychotic symptoms during the scan were averaged for the control and experimental part, and the difference score (experimental-control) was used as the outcome variable in regression analyses, with childhood trauma scores entered as predictors. To compare the two groups on the change in perceived difficulty of the task, this variable was averaged for the control and experimental part, the difference score was computed as above, and entered in a regression analysis as the outcome, with group (NAPD, HV) as the predictor. Regression analyses were corrected for age and gender.

RESULTS

Sample Demographics

As decribed in detail previously (25), HV and NAPD were matched on age (M=48.08 (SD=9.94) vs. M=44.67 (SD=11.24)), gender (8 male, 4 female per group), education, lifetime drug use and smoking frequency (all n.s.). Four NAPD were antipsychotics (AP) –naïve; the remainder of the sample were off AP for 7.09 (SD=4.96) years. Patients endorsed moderate levels of positive symptoms of psychosis (PANSS positive symptom scale mean = 11.83, SD= 3.93). The ability of the MIST to successfully induce stress and temporarily increase positive psychotic symptoms in this sample has been reported previously (25). Additionally, the ratings of the perceived difficulty of the task increased numerically from control to experimental condition for both HV (M=1.63, SD=1.76) and NAPD (M=1.54, SD=1.40) to the same extent (b=-.17, t(1,23)=-.25, p=.807). Healthy participants endorsed a mean of 2.42 (SD=1.51) adverse events in early childhood and 2.67 (SD=1.5) adverse events in late childhood. NAPD scored 3.42 (SD=1.88) and 2.75 (SD=1.36) for early and late childhood trauma respectively. The two groups did not differ in early (t(1,23)=-1.31, p=.20) nor late childhood trauma scores (t(1,23)=-.29, p=.77).

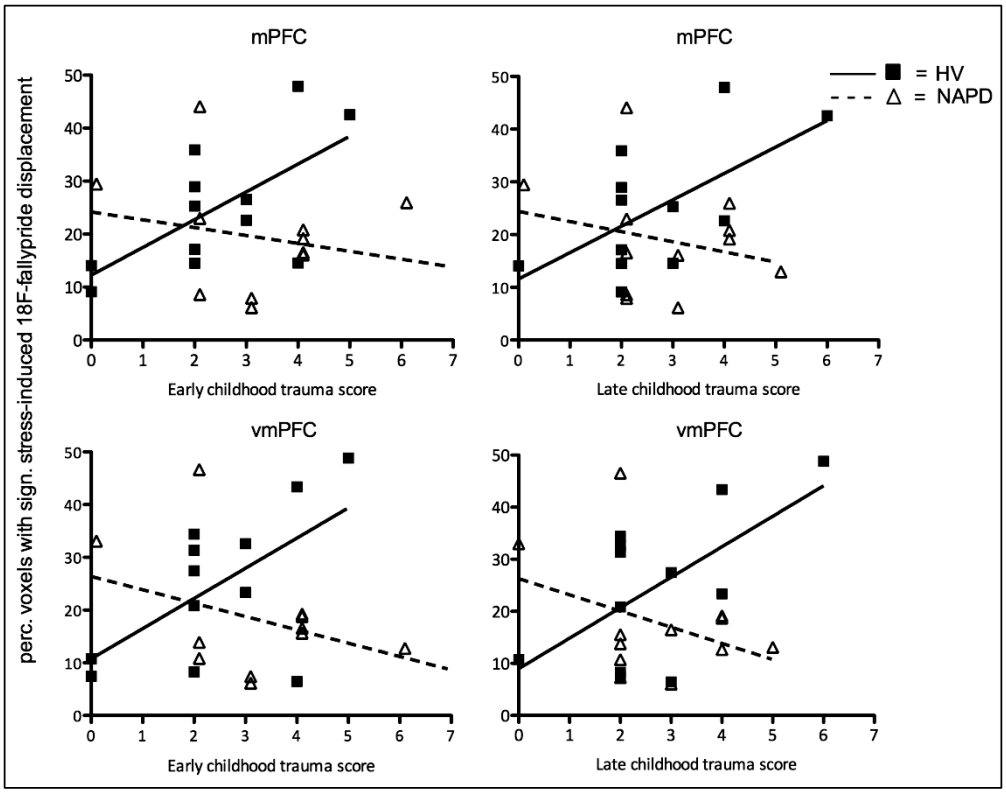
The effect of childhood trauma on stress-induced [^{18}F]fallypride displacement

Binding potential relative to non-displaceable binding potential (BP_{ND}) calculated over the complete paradigm using the SRTM (40) in mPFC was .51 (SD=.2). As evidenced by Fig. 2, a significant group x childhood trauma interaction on the spatial extent of stress-related [^{18}F]fallypride displacement was observed in the mPFC for early childhood trauma (b=-8.45, t(1,23)=-3.35, p=.004), and late childhood trauma (b=-7.86, t(1,23)=-2.48, p=.023). Within the control group, a significant positive association emerged between the spatial extent of stress-induced tracer displacement in the mPFC and early childhood trauma (b=7.23, t(11)=3.06, p=.016; Fig. 2 and 3) and late childhood trauma scores (b=5.47, t(11)=2.54, p=.035; Fig. 2). In the patient group, there was no association between childhood trauma and the spatial extent of the tracer displacement in mPFC (early b=-1.22, t(11)=-0.67, p=.519; late b=-1.68, t(11)=-.68, p=.513; Figure 2 and 3.

An analogous significant group x childhood trauma interaction effect was observed in the vmPFC (early b=-9.53, t(1,23)=-3.15, p=.006; late b=-9.5, t(1,23)=-2.61, p=.018; Fig. 2). In healthy controls, a trend-level positive association between the spatial extent of stress-induced tracer displacement in the vmPFC and childhood trauma was observed for both early trauma (b=7.2, t(11)=2.22, p=.058) and late trauma scores (b=6.02, t(11)=2.24, p=.056). Similarly to the mPFC, there was no significant association between tracer displacement in the vmPFC and childhood trauma in the patient group

(early b=-2.18, t(11)=-1.15, p=.282; late b=-2.7, t(11)=-1.03, p=.331; Fig. 2). There was no main effect, interaction effect or within-group associations between the two childhood trauma scores and stress-related fallypride displacement in the dmPFC (all p-values>.05).

Figure 2: The effect of childhood trauma on spatial extent of DA activity in mPFC and vmPFC



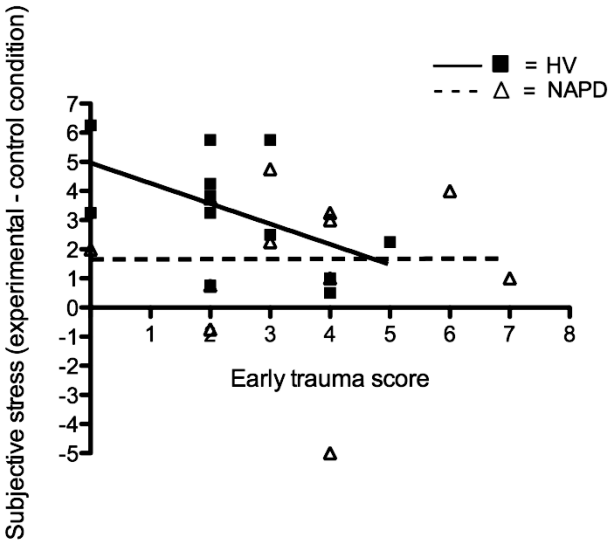
Early (ages 0-11) and late (ages 12-17) childhood trauma scores (x-axis) were associated with increased spatial extent of stress-induced mPFC and vmPFC [^{18}F]fallypride displacement (y-axis) in HV. No such associations were observed in NAPD. Association in HV significant at p<0.05.

The effect of childhood trauma on subjective stress and psychopathology

The association between early trauma score and subjective stress scores during the MIST paradigm was not significantly different for NAPD and HV (b=.7, t(1, 23)=1.18, p=.26). However, when testing within-group main effect of childhood trauma on subjective experience of stress, a trend for a negative association between early childhood trauma and the subjective stress response was detected in HV (b=-.7, t(11)=-2, p=.07), with higher early childhood trauma being associated with lower subjective

stress responses to the task (Fig. 3). No main effect of childhood trauma on the subjective stress response to the task was present in NAPD (b=.004, t(11)=.01, p=.99; Fig. 3). There was no interaction or main effect between late childhood trauma and the subjective stress response to the task (all p-values>.05) and there was no association between the two childhood trauma scores and psychotic symptoms during the scan in NAPD.

Figure 3: Correlation between early childhood trauma scores and subjective stress during PET



A trend-level association between early childhood trauma scores (0-11) and decreased reactivity to the stress task in HV, but not NAPD. Association in HV p=0.07.

DISCUSSION

We examined the association between childhood trauma and stress-induced prefrontal DA activity of healthy individuals and patients with psychotic disorder using [^{18}F]fallypride PET. We observed a significant difference in the association between childhood trauma score and spatial extent of stress-induced prefrontal DA activity in each group; In healthy subjects, severity of childhood trauma was associated with more extensive stress-related DA activity in mPFC. This effect was especially pronounced in relation to early childhood trauma, and largely driven by DA activity in the ventral portion of mPFC. Contrarily, in the patient group, there was no association between childhood trauma and the spatial extent of stress-related DA activity in this region, and this was the case for its ventral and dorsal portions, as well as for early and late childhood trauma. While the interaction between group and childhood trauma on

behavioral stress response was not significant, a trend for a main effect of early trauma emerged in the control group, where increased exposure to early trauma was associated with decreased subjective stress responses to the task. No main effect of childhood trauma on subjective experience of stress was detected in individuals with psychotic disorder.

Healthy individuals

These results first of all implicate prefrontal DA transmission in the human stress response and confirm the role of the mPFC in this function. Furthermore, they build upon our previous findings of increased DA activity in mPFC under acute psychosocial stress in this (25) and another sample (30), by showing that in the healthy brain, distal forms of stress impact the acute prefrontal DAergic stress response. The results presented in this manuscript suggest that increased DAergic activity observed in the striatum of those exposed to childhood adversity (28) also extends to the cortex.

The positive association between childhood trauma and the spatial extent of mPFC DA activity under stress in healthy adults could be interpreted as one of the mechanisms of adaptive neuroplasticity in the mPFC (16), characterizing resilience. The within-group behavioral results suggest that this mechanism may underlie increased robustness to psychopathology, as more severe trauma was associated with decreased sensitvity to the experimental stressor.

This notion corroborates the emerging evolutionary perspective on resilience to psychopathology which maintains that early life adversity could induce adaptive changes that optimize the individual's fit with the predicted (adverse) environment (13). That is, stress during development could "inoculate" certain individuals to better cope with challenges encountered during adulthood (7), such as those evoked by the present experiment.

While social support, parenting and other external circumstances undoubtedly play a role, genetic makeup is thought to largely determine a stress-vulnerable versus stress-resilient phenotype (8). Neuroadaptation to stress is multifold, and believed to involve variation in the glucocorticoid receptor (GR; (41) and catechol-O-methyltransferase (COMT) expression, both of which directly influence prefrontal DA function (42). Stress has been shown to exclusively activate the GRs located on mPFC DA neurons leading to DA efflux, which in turn mediates DA release downstream (43). In the interaction with childhood trauma, common variants of the GR gene predict increased biomarkers for, and actual vulnerability to, psychopathology in adulthood (44). Meanwhile, COMT genotype predicts the extent of prefrontal DA activity and stress-sensitivity (38), and is reported to modulate the effect of childhood trauma on cognition and symptoms of psychosis (45). Collectively, these studies offer one possible explanation of how childhood adversity in interaction with (epi)genetically optimized prefrontal DA reactivity to stress may confer a resilient phenotype.

Individuals with psychotic disorder

In NAPD, on the other hand, there was no association between childhood trauma and the spatial extent of stress-related DAergic activity in this region, and this was the case for its ventral and dorsal portions, as well as for early and late childhood trauma. Moreover, no within-group association between childhood trauma and subjective stress response to the task was observed. Seeing that this pattern deviates from the putative adaptive DA response of HV, one could reasonably speculate that in individuals that develop psychosis later lin life, childhood trauma fails to evoke the necessary calibration of the DA system to better endure stress (8). From the large-scale brain network perspective, the dysregulation of the prefrontal node by childhood trauma could have a noxious effect on stress responsiveness in the interconnected subcortical hubs (46). This notion is supported by reports from other groups implicating the striatum in aberrant reactivity to stress in psychosis in general (27) and in the pathogenesis of psychosis in particular (47, 48). Furthermore, low maternal care in the early life has been shown to be more prevalent in individuals with schizotypy and associated with increased stress-induced DA release in the striatum (29).

The underlying mechanism of such divergent trajectories of individuals with psychosis exposed to similar levels of childhood trauma as their healthy counterparts could be attributable to a stress-susceptible genetic make-up. In addition to studies implicating GR and COMT variants in poor outcomes following childhood trauma discussed earlier, an extensive general population study identified the COMT polymorphism as significant moderator of the susceptibility to psychotic experiences following childhood maltreatment (49). Meanwhile, patients with psychosis carrying the COMT Met/Met genotype demonstrated increased affective and psychotic reactivity to stress (50). Collectively, these studies support the existence of a stress-vulnerable genotype implicating prefrontal DA function and warrant integrated exploration of the trauma-stress-psychosis connection.

Strengths and Limitations

A number of strengths and limitations regarding the design and methodology of the current study were previously discussed in detail elsewhere(25).

The most important consideration includes the fixed order of the control-experimental condition to accommodate the model and prevent the long-lasting effects of stress from contaminating the control condition. It is possible that this design could introduce an order effect due to, for instance, greater proneness to fatigue in the patient group. Although a recent [^{18}F]fallypride PET experiment that employed the MIST reported a main effect of stress on mPFC DA activity irrespective of the condition order (26), the two conditions were administered on separate days, and the population under study only included healthy controls. While in the current study patients did

not endorse greater increase in perceived difficulty of the stress than the controls did, it still does not rule out the possibility that they were more fatigued by it or more reactive to it in some other way.

Other limitations specific to this article include the variation in BD present in both groups that suggests that although the DA system plays an important role, other factors likely also contribute to resilience and vulnerability to stress. Additionally, the relatively small sample size could both preclude and inflate the subtle effect of childhood trauma on the arguably noisy DA-ergic neurotransmission of the 12 patients included in this study. According to our post-hoc power calculation, doubling this sample size would yield moderate-to-high power in future exploration of this intriguing phenomenon.

Another limitation pertains to the CECA questionnaire used to quantify childhood trauma. The retrospective nature of the self-report of stressful childhood experiences is subject to recall bias. However, this questionnaire has been well-validated and widely-accepted as an accurate and reliable index of exposure to adversity in childhood (51, 52). Moreover, the structure of the CECA questionnaire used in the current study only allows for quantification of the stressful life events, but does not allow to qualify the frequency and gravity of the event self. This compromise has been introduced in order to minimize memory bias, as it has been shown that the recollection of an event is more susceptible to forgetting and false memory formation than a mere recognition of a presence or absence of the event (53, 54).

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Chapter 7

The anatomy of fear learning in the cerebellum: A systematic meta-analysis

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ABSTRACT

Recent neuroimaging studies have implicated the cerebellum in several higher-order functions. Its role in human fear conditioning has, however, received limited attention. The current meta-analysis examines the loci of cerebellar contributions to fear conditioning in healthy subjects, thus mapping, for the first time, the neural response to conditioned aversive stimuli onto the cerebellum. By using the activation likelihood estimation (ALE) technique for analyses, we identified several distinct regions in the cerebellum that activate in response to the presentation of the conditioned stimulus: the cerebellar tonsils, lobules IV-VI, and the culmen. These regions have separately been implicated in fear acquisition, consolidation of fear memories and expression of conditioned fear responses. Their specific role in these processes may be attributed to the general contribution of cerebellar cortical networks to timing and prediction. Our meta-analysis highlights the potential role of the cerebellum in human cognition and emotion in general, and addresses the possibility how deficits in associative cerebellar learning may play a role in the pathogenesis of anxiety disorders. Future studies are needed to further clarify the mechanistic role of the cerebellum in higher order functions and neuropsychiatric disorders.

INTRODUCTION

The cerebellum has traditionally been predominantly implicated in motor control and coordination. However, many recent accounts have been providing evidence for a role of the cerebellum in higher order functions.

Animal studies and functional connectivity MRI studies in humans have shown that, via cortico-ponto-cerebellar and cerebello-thalamo-cortical loops, the majority of cerebellum projects to many cerebral association and limbic areas, including the prefrontal and parietal cortex, amygdala, hippocampus, hypothalamus, striatum, and brain stem (1-4). Therefore, it is not surprising that lesions of the cerebellum not only result in prominent motor symptoms, but also in impairments in executive functioning, spatial cognition, language, and changes in personality and affect (5). Consequently, in 1998, this cluster of symptoms received the name cerebellar cognitive affective syndrome (6, 7). Furthermore, structural and functional abnormalities of the cerebellum have also been associated with impaired mood regulation and cognitive functioning in a variety of psychiatric conditions including autism, anxiety disorders, depression and psychosis (8, 9). Finally, recent studies map various higher order processes, such as executive functioning, language, spatial and emotional processing, to distinct regions of the cerebellum (10-13).

Preclinical studies have pointed towards a role of the cerebellum in different forms of associative learning. Data from animal studies suggest that the cerebellum is involved in motor learning, such as eyeblink conditioning (14-17) and adaptation of the vestibulo-ocular reflex (18, 19). However, several experimental animal studies predominantly employing the classical fear conditioning paradigm also implicate the cerebellum in emotional learning (20). Lesion studies refine these findings by showing that lesions of the vermis result in impaired acquisition and retention of fear-conditioned autonomic responses as well as in attenuation of fear-related behaviors (21, 22). Furthermore, blockade of the cerebellar vermis after fear learning produces amnesia, which has been interpreted as interfering with storage and/or memory trace retrieval. In addition, fear learning has been shown to induce long-term potentiation (LTP) in parallel fibers to Purkinje cells in vermal lobules V-V1 (23, 24). LTP in these areas is assumed to be related to the consolidation of fear memories (25), akin to the function of an LTP mechanism that takes place in amygdala and hippocampus (26, 27). However, a recent study in cerebellar mouse mutants has shown that impairments in Purkinje cell plasticity did not affect fear responses during both cued and contextual conditioning (28), even though it did result in learning deficits when a cognitive task with temporal constraints was employed (29). These results suggest that the cerebellum is essentially concerned with tasks requiring precise temporal accuracy (29, 30).

Similar to the animal literature, the role of the cerebellum in human fear learning has received relatively limited attention. Possibly, the role of the cerebellum in higher

order processes is more pronounced in the human than in the murine cerebellum (28), which could be a consequence of the enlargement of the ventral dentate nucleus and related cerebellar cortical hemispheric regions, paralleling the enlargement of the prefrontal cortex (31, 32). Even though the cerebellum has been pointed out as one of the regions often activated in human fear-conditioning paradigms (33), few studies to date have explored which specific cerebellar regions are involved in human fear learning (34, 35). Lesion studies and fMRI studies of healthy individuals suggest a role of the vermis in fear-conditioned potentiation of motor and autonomic responses (36, 37), whereas activation in left lobule V1 is proposed to be associated with the acquisition of fear (34). Furthermore, many fear learning paradigms involve sensorimotor and timing components next to emotional learning (29), thus further obfuscating the investigation into the primary role of the cerebellar regions in the purely emotional, non-motor aspects of the fear learning process. As a result, a decisive account of the precise regions and functional contribution of the cerebellum involved in human fear learning remains to be determined.

Aim of the present meta-analytic study is to examine the results of all available human fMRI studies in a systematic fashion, and thus shed light on the precise location of the cerebellar contributions to fear learning in the healthy population. Therefore, in order to unequivocally determine the locus of cerebellar activity associated with fear learning we gathered, reviewed and analyzed all published functional magnetic resonance imaging studies on this subject.

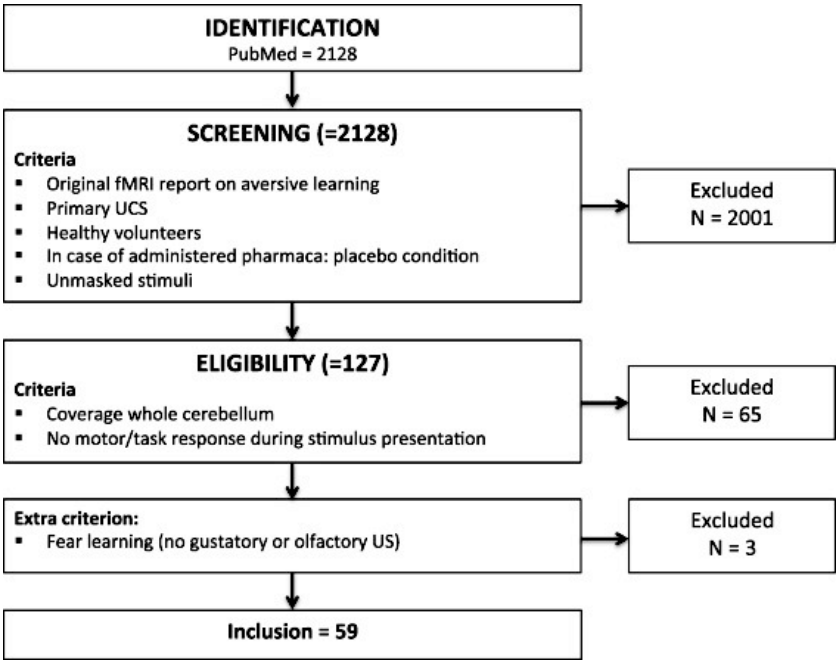
MATERIALS AND METHODS

Step 1: Literature Review

Two researchers (IL, ZK) independently performed the search, screening, selection and coding steps of this meta-analysis. The PubMed database was searched for words 'emotional learning' or 'aversive learning' and additionally for 'fear conditioning or 'fear learning' AND 'imaging', with the filter for, 'human'. Articles published until December 2013 were screened on titles, abstracts and/or full texts: the search revealed in total 2128 articles. The following criteria for the inclusion of articles were selected a priori. First, the article had to satisfy the criterion of being an original report of an fMRI study of learning from emotional stimuli in healthy volunteers. For these purposes, emotional stimuli are defined as neutral stimuli that acquire emotional salience through consistent pairing with aversive outcome. Learning is defined as the subjects' acquisition of the conditioned response to the stimuli. Our inclusion criteria were further limited to paradigms that delivered primary unconditioned stimulus (UCS), such as electroshock or noise. Second, studies that employed masking of the stimuli, administered a drug without a placebo condition or investigated clinical population without

healthy control group were excluded. However, whenever data from a placebo condition or healthy control group were available, we included them into our analyses. For studies that had overlapping subject groups, we only included the study with the largest sample size. In case these data were not specified in the full text, authors were contacted. A total of 127 articles were included. Full texts of these articles were screened for three additional criteria. First, only studies in which the entire cerebellum was covered were included. In cases where it was not specified in the article that the whole brain was covered during the fMRI, the authors were contacted to ascertain this. Second, studies that required a motor response or task during CS presentation were eliminated. Lastly, in order to maintain focus on fear learning rather than aversive learning, studies that made use of gustatory or olfactory unconditioned stimuli were excluded. Finally, 59 articles were included. See figure 1 for the decision tree.

Figure 1: Decision tree



Step 2: Description of the studies

The 59 included articles were reviewed in detail and characterized based on the population under study, experimental paradigm, modality of the presented stimuli and valence of the stimuli. 49 studies tested healthy volunteers only and 10 investigated clinical populations in conjunction with healthy controls. 45 articles employed differen-

tial fear conditioning paradigms only, 9 used fear conditioning followed by extinction, 2 reported fear conditioning with reversal and 3 with generalization. Visual presentation of the CS (neutral image) paired with tactile UCS (electroshock) was the prevailing modality, reported in 46 studies, followed by visual CS with auditory UCS (loud tone) in 7 studies, auditory CS (neutral tone) with auditory UCS (loud tone) in 3 studies, auditory CS (neutral tone) with tactile UCS (electroshock) in 2 studies, and olfactory CS (neutral odor) paired with tactile UCS (electroshock) were administered in 1 study. While all paradigms employed aversive outcome after the CS (CS+), these were contrasted with no UCS in 55 of the studies and non-noxious UCS in 4 studies following the other CS (CS-).

The main outcome measure was the coordinate of significant cerebellar activation associated with fear learning. As different studies used different methodologies, we extracted coordinates regarding the response to the conditioned stimulus (CS+) predicting the fearful event, including the contrasts 'CS+>CS-' and 'CS+>Baseline'. These coordinates were originally reported in 19 articles. We requested the missing data, including negative findings, from corresponding authors of the remaining 40 articles via email. The response rate was 75%. Our final data pool consisted of 21 articles reporting cerebellar activation, 26 accounts of no detected activity, 5 reports of unavailable data sets, and 7 articles in which only data for other regions of interest were reported. The articles included in the final analyses provided a total of 43 coordinates of cerebellar activity associated with fear learning. Two studies reported coordinates regarding the response to the CS+ versus baseline; the other 19 studies reported coordinates regarding the contrast CS+ versus CS-. The total number of subjects included was 614 (see Table 1).

Table 1: All included studies listed by first author. Coordinates are given in Talairach space.

First Author	Year	N	Valence (CS+, CS-)	Modality (CS+ (UCS)	x	y	z	Field strength
Cacciaglia	2013	114	aversive, none	visual (tactile)	-13	-75	-30	1.5T
Cacciaglia	2013	114	aversive, none	visual (tactile)	-29	-59	-29	1.5T
Cacciaglia	2013	114	aversive, none	visual (tactile)	-35	-45	-42	1.5T
Cacciaglia	2013	114	aversive, none	visual (tactile)	-32	-65	-29	1.5T
Cacciaglia	2013	114	aversive, none	visual (tactile)	-35	-58	-40	1.5T
Cacciaglia	2013	114	aversive, none	visual (tactile)	-17	-72	-30	1.5T
Cacciaglia	2013	114	aversive, none	visual (tactile)	28	-59	-28	1.5T
Cacciaglia	2013	114	aversive, none	visual (tactile)	28	-49	-38	1.5T
Carlson	2011	35	aversive, none	visual (auditory)	-21	-62	-18	3T
Carlsson	2006	9	aversive , non- aversive	visual (tactile)	-10	-46	-16	1.5T
Carlsson	2006	9	aversive , non- aversive	visual (tactile)	-7	-46	-11	1.5T
Cheng	2008	11	aversive, none	auditory (tactile)	-24	-57	-23	3T
Coen	2011	31	aversive, none	visual (tactile)	29	-59	-20	3T
Coen	2011	31	aversive , none	visual (tactile)	-29	-52	-26	3T
Delgado	2008	12	aversive , none	visual (tactile)	25	-29	-17	3T
Delgado	2009	32	aversive , none	visual (tactile)	-33	-52	-23	3T
Delgado	2009	32	aversive , none	visual (tactile)	31	-43	-21	3T
Jensen	2003	11	aversive, none	visual (tactile)	18	-43	-36	1.5T
Kalish	2006	15	aversive , none	auditory (tactile)	1	-49	-17	3T
Kalish	2006	15	aversive , none	auditory (tactile)	-19	-45	-36	3T
Kattoor	2014	30	aversive , none	visual (tactile)	22	-48	-35	3T
Kattoor	2014	30	aversive, none	visual (tactile)	-42	-44	-30	3T
Kattoor	2014	30	aversive , none	visual (tactile)	44	-48	-23	3T
Kattoor	2014	30	aversive , none	visual (tactile)	-30	-35	-27	3T
Kattoor	2014	30	aversive, none	visual (tactile)	-35	-44	-43	3T
Kattoor	2014	30	aversive , none	visual (tactile)	35	-50	-39	3T
Knight	2005	9	aversive , none	auditory (auditory)	15	-50	-24	1.5T
Olsson	2007	11	aversive , none	visual (tactile)	5	-50	-18	3T
Olsson	2007	11	aversive , none	visual (tactile)	39	-55	-22	3T
Ploghaus	1999	12	aversive , non- aversive	visual (tactile)	-7	-62	-21	3T
Ploghaus	2000	10	aversive , non- aversive	visual (tactile)	-24	-66	-26	4T
Ploghaus	2000	10	aversive , non- aversive	visual (tactile)	23	-63	-25	4T
Pohlack	2012	131	aversive , none	visual (tactile)	-39	-55	-36	1.5T
Schiller	2008	17	aversive , none	visual (tactile)	14	-42	-15	3T
Spoormaker	2011	40	aversive , none	visual (tactile)	29	-39	-21	1.5T
Spoormaker	2011	40	aversive , none	visual (tactile)	-34	-47	-15	1.5T
Strigo	2008	15	aversive , non- aversive	visual (tactile)	4	-49	-17	3T
Strigo	2008	15	aversive , non- aversive	visual (tactile)	7	-71	-22	3T
Veit	2002	7	aversive , none	visual (tactile)	-37	-45	-47	1.5T
Visser	2013	54	aversive , none	visual (tactile)	-33	-55	-36	3T
Yaguez	2005	8	aversive , none	visual (tactile)	-4	-52	-29	1.5T
Yaguez	2005	8	aversive , none	visual (tactile)	17	-67	-13	1.5T
Yaguez	2005	8	aversive , none	visual (tactile)	-7	-86	-24	1.5T

Table 2: Characteristics of the fear conditioning studies included in this meta-analysis

Author, year	Reinforcement	CS-US delay	Objective readouts	Subjective measures	Statistical Thresholds
Cacciaglia, 2013	50%	Co-termination (CS 6s; US last 2.7s)	SCR	Arousal, valence, contingency awareness	$p < 0.05$ (FWE corrected)
Carlson, 2011	50%	16s delay between CS-US	-	Anxiety	$p < 0.05$ (FDR corrected)
Carlsson, 2006	100%	No delay between CS-US	-	Anxiety, valence, pain intensity	$p(beta > 0 \mid y) > 0.975$
Cheng, 2008	100%	Delay conditioning: co-termination (850ms CS; last 100ms US) Trace: 500 ms delay between CS-US	Eyeblink response	Contingency awareness	$p < 0.025$ (in cerebellum ROI)
Coen, 2011	100%	3-12s delay between CS-US	SCR	Perception of pain	$p < .003$ (corrected)
Delgado, 2008	55%	Co-termination (CS 4s; US last 200ms)	SCR	-	$p < 0.005$
Delgado, 2009	100%	3.8-5.8 delay between CS-US (response phase in between)	SCR	Contingency awareness	$p < 0.01$ (FDR corrected)
Jensen, 2003	100%	1.74-1.9s delay between CS-US (target phase in between)	SCR	-	$p < 0.001$ (uncorrected)
Kalish, 2006	25%	0-15.6s delay between CS-US	Heart rate	Time feeling anxious, time spent thinking about pain or anxiety	$p < 0.001$
Kattoor, 2014	75%	Co-termination (CS 7.2-12s)	-	Contingency awareness, valence	$p < 0.001$ (uncorrected)
Knight, 2005	100%	Co-termination (CS 10s; US last 500ms)	SCR	-	$p < 0.01$ (?)
Olsson, 2007	60%	Co-termination (CS 10s)	SCR	-	$p < 0.001$, uncorrected
Ploghaus, 1999	100%	Co-termination (CS ± 18.5s; US last 11s)	-	pain intensity, unpleasantness	$p < 0.05$

Author, year	Reinforcement	CS-US delay	Objective readouts	Subjective measures	Statistical Thresholds
Ploghaus, 2000	100%	Co-termination (CS ± 18.5s; US last 11s)	-	Contingency awareness, pain intensity, unpleasantness	$p < 0.05$ (FWE corrected)
Pohlack, 2012	50%	3-10s delay between CS (full intensity) – US	SCR	Contingency, valence, arousal	$p < 0.05$ (FWE corrected)
Schiller, 2008	30%	Co-termination (CS 4s; US last 200ms)	SCR	-	$p< 0.05$ (FDR corrected)
Spoormaker, 2011	50%	3.1s delay between CS-US	SCR	-	$p < 0.025$ (FDR corrected)
Strigo, 2008	100%	No delay between CS-US	-	Average intensity and unpleasant- ness of US	$p < .05$ (corrected)
Veit, 2002	100%	No delay between CS-US	SCR)	Valence, arousal, contingency awareness	$p < 0.001$ (uncorrected)
Visser, 2013	50%	Co-termination (CS 4.5s)	Pupil dilation response	US expectancy scores	$p < 0.05$ (cluster corrected)
Yaguez, 2015	100%	Co-termination (CS3s; US last 500 ms)	-	-	$p < 0.001$ (uncorrected)

Step 3. ALE Meta-analysis

In order to determine the locus of cerebellar activation in response to fear learning, we performed a coordinate-based meta-analysis of functional imaging data using the activation likelihood estimation (ALE) procedure, facilitated by the GingerALE 2.3.1 software developed by BrainMap (www.brainmap.org/ale). This tool is designed to incorporate the coordinates reported in multiple experiments into one convergent focus. ALE analysis is based on the assumption that the foci reported in neuroimaging studies are not single points, but rather spatial probability distributions centered at the given coordinates. The resulting maps represent the union of activation probabilities for each voxel, when the null hypothesis that their convergence is random is rejected via a permutation procedure (38). Instead of using pre-specified full-width half maximum (FWHM) for all experiments, the probability distribution of each focus is modeled using an estimation of the inter-subject and inter-study variability present in standard neuroimaging studies (39). This analytical method thus takes into account the sample size of each contributing study and results in a pooled, uniform final cluster locations labeled anatomically according to the Talairach atlas (39-41).

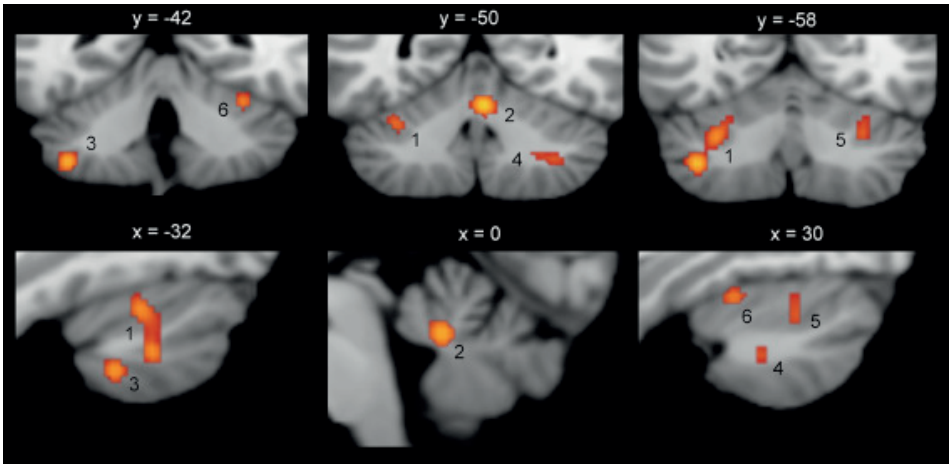
First, we extracted a total of 43 coordinates corresponding to sub-threshold cerebellar activity reported in the 21 included studies. Prior to serving as an input for the GingerALE tool, the MNI coordinate systems determined by the SPM software were converted to Talairach coordinates using the icbm_spm2tal transform. The Talairach coordinates calculated by the SPM software using the Brett method were converted back into their original MNI space using the tal2mni transform, followed by the icbm_spm2tal to convert them into the more suitable Talairach coordinates. Next, the coordinates were entered into the GingerALE application with a p-value specified at < 0.05 for false discovery rate and minimum cluster size of 100 mm^3. The two main outcomes of interest are the p-values at each voxel, and the ALE map containing the significant clustered voxels. This map was then overlaid onto the anatomical Talairach template and displayed in axial orientation using Mango, multi-image viewing software (www.ric.uthscsa.edu/mango). The anatomical labels for the three ALE maxima were obtained using Talairach Client version 2.4.2 supplied by Talairach Daemon (Talairach.org).

RESULTS

The results of the ALE meta-analysis of the 21 studies on fear learning revealed six clusters of significant activation-likelihood located in the cerebellum. The activation found was roughly symmetrical across the cerebellar hemispheres. The largest peak encompassed left lobules HIV-V, HVI, and, lobule HIX. The second peak was found in the culmen. Other peaks were found in the left and right lobules HIX and right lobules

HIV-V. Table 3 shows the peak coordinates, cluster sizes, peak ALE values and the weighted centers. Figure 2 shows the locations of the peak coordinates in both coronal and sagittal views.

Figure 2: The clusters of significant activation-likelihood in the cerebellum associated with fear learning



The images are shown in neurological orientation; the left side of the image corresponds to the left side of the brain. 1= left HIV-V-VI-IX; 2= culmen; 3= left HIX; 4= right HIX; 5= right HIV-V; 6= right HIV-V

Table 3: Peak ALE values for six clusters significant at FRD (False Discovery Rate) $p < 0.05$. All coordinates are in Talairach space.

Cluster	Anatomical label	x	y	z	Volume (mm3)	ALE value (x 10-3)	Weighted centre (x, y, z)		
1	Left HIX	-36	-56	-38	1776	20.8	-31	-56	-30
	Left HIV-V	-30	-54	-26		17.1			
	Left HVI	-22	-60	-20		12.2			
2	Culmen	4	-50	-18	576	19.2	3	-49	-17
3	Left HIX	-36	-44	-42	568	21.3	-35	-44	-43
4	Right HIX	24	-48	-36	424	13.4	26	-48	-37
		30	-50	-38		12.8			
5	Right HIV-V	28	-60	-26	336	13.9	27	-60	-25
6	Right HIV-V	30	-40	-20	240	15.0	30	-41	-21

DISCUSSION

The aim of this meta-analysis was to synthetize findings from fMRI studies reporting cerebellar activation during fear conditioning in healthy individuals in order to decisively map the neural response associated with conditioned stimulus onto the cerebellum. The results of this ALE meta-analysis show six specific cerebellar regions involved

in fear learning: the culmen, right and left lobule HIV-V and left lobule HVI, and right and left lobule HIX.

The largest peak encompassed the lobules HIV-V, HVI, and HIX. Converging evidence from human in-vivo neuroimaging studies implicate these regions in both classical conditioning and associative learning process (35, 42, 43). The involvement of lobule HVI in the acquisition and memory of the conditioned nictitating membrane response or eyeblink response has been confirmed by both animal and human imaging studies (44-46). Animal studies show that lesions or inactivation of lobule HVI result in impaired fear learning rates, while the expression of the unconditioned responses remains intact (47-49). This suggests that lobule HVI plays a role in memory formation, but not in the preparation or execution of the eyeblink response per se. Both LTD and LTP in this region have been suggested as possible mechanisms of cerebellar learning (19, 50, 51), but recent mouse mutant studies point towards a stronger role for LTP than LTD (52-54). Imaging studies of fear conditioning additionally link lobule HVI with the learning process during fear conditioning (34, 35). It is noteworthy, however, that this region has also been implicated in working memory, executive functioning, spatial processing and language (12).

Lobule HIV-V, on the other hand, has previously not been specifically pointed out as one of the regions involved in fear learning, although it has been shown to contribute to late extinction learning and reinstatement (35, 42). Activation in this area has also been observed during instances of negative prediction error (55, 56), a critical component of aversive learning. Importantly, this region has also been linked to reward anticipation (57, 58), extending its putative role to learning from both negative and positive outcomes. Furthermore, a meta-analytic study confirmed hat both the left and right amygdala are functionally connected to lobules HIV-V (59). In addition, both animal and human studies have shown that this area is also involved in the timing of conditioned motor responses (14, 60, 61). Thus, lobules HIV-V might be involved in valence, prediction and timing of motor and sensory events during emotional learning processes.

Several peaks were found in lobules HIX, the cerebellar tonsils. A recent study by Kattoor and colleagues (35) found that these regions are involved in extinction learning and reinstatement. It has been suggested that the cerebellar tonsils have a role in cognition, as they have consistently been found to be part of the default mode network (62-64). Importantly, they have been shown to play a role in working memory, recognition memory, and mechanisms of reward anticipation (65-67), as well as in the perception of change in stimulus timing (68). Critically, these areas were also activated during processing of negative stimuli and during the experience of fear and anger (69, 70). Therefore, we suggest that the cerebellar tonsils might be involved in working memory, and more specifically, in contingency and valence learning. This would be in line with previous studies suggesting that the vermis is involved in fear-conditioning

related somatic responses, while the postolateral hemispheres may play a role in emotional and cognitive associative learning (20, 70).

A second large peak was found in the culmen, a part of the anterior vermis. Although this area has traditionally been associated with sensorimotor processing, several studies have demonstrated that it also contributes to higher order functions such as verbal learning and memory, social cognition, emotional processing, and the expression of emotional behaviour (71-73). The anterior vermis has efferent connections with the limbic formations, including the amygdala, hippocampus, nucleus accumbens, septal regions, and orbitofrontal cortex (74, 75), all off which are involved in emotional learning. Furthermore, it has reciprocal connections to the hypothalamic-pituitary-adrenal (HPA) axis (76). Importantly, animal studies have previously shown that electrical stimulation of this area elicits behavior consistent with fear and anxiety (77), while inactivation of this region results in impairment of these responses (22, 23). Further examination confirmed that the vermis, and more specifically the culmen, contributes to the acquisition, expression and memory of fear conditioning-related autonomic responses (21, 23, 78). Corroborating histological evidence from rodent studies revealed synaptic changes in the vermal lobules HIV-V following fear conditioning, a process thought to constitute the neural substrate of the fear memory formation (21, 24). Of note, fear conditioning-related LTP commonly occurring in amygdala and hippocampus was also detected at the synapses of parallel fibers to Purkinje cells in the locus of peak activation identified in the present meta-analysis (24, 54). The animal literature has suggested that the anterior vermis is part of a conditioned emotional response due to its involvement in the integration of the valence of sensory stimuli and conditioned motor and autonomic responses (64, 78). Moreover, a human PET imaging study and human brain lesion studies have also implicated this cerebellar area in fear-conditioned potentiation (20, 34, 36, 37).

Our results are partially in agreement with an experimental fMRI study focusing specifically on cerebellar involvement in fear learning. However, this study also showed additional activations (35) in other regions, including lobules Crus 1, right HVIIB, right Crus II, and parts of the dentate nucleus (35). No activation peaks in these areas were found in the current meta-analysis. This could be explained by the strictness of the threshold of statistical significance introduced by the correction for multiple comparisons necessary when pooling the results of multiple studies, as was the case in this meta-analysis. Arguably, the inclusion of multiple studies, and the stringent statistical adjustments, may lead to more robust findings.

Since the cerebellum as a whole has been shown to play a role in several types of associative learning mechanisms including motor, cognitive, and emotional learning, it has been hypothesized that the cerebellum contributes to the prediction and timing of sensory and motor events (20, 79). More specifically, the cerebellum is thought to be mainly involved in temporal processing and predictive coding of events by integrating different types of signals (80-82), thereby forming a domain-general, but temporally

specific learning mechanism (81, 83). Several regions have been shown to be involved in temporal processing, including lobules IV-V, VI, VII, VIIIa, and the superior vermis (60, 84, 85). Within fear conditioning paradigms, precise timing and predictive action are essential. It is therefore plausible, although still speculative, that fear learning might depend on cerebellar common computations of basic functions such as timing, predictive action and sequence learning embedded in connections with other brain regions belonging to the fear circuitry. If cerebellar involvement in fear conditioning reflects temporal prediction of events (30), it would likely contribute to other forms of associative learning such as eyeblink conditioning. In eyeblink conditioning, a conditioned stimulus is coupled with an eyeblink-eliciting stimulus, such as an air puff or a shock, in a manner that parallels the procedures employed in fear conditioning. Studies of eyeblink conditioning have indeed found involvement of several cerebellar regions, including lobules HIV-IX and vermis IV-VI (46, 86). The role of these regions in learning or performance of the eyeblink response is still under question (87). Although fear conditioning and eyeblink conditioning overlap procedure-wise, temporal differences in the output exist: eyeblink conditioned responses emerge after numerous CS-US pairings, while conditioned fear responses are present after only a couple CS-US parings (88). A two-process model of conditioning suggests that emotional learning and fear responding precede and subsequently facilitate conditioning of the motor response (88) (89).

If cerebellar involvement in emotional learning reflects temporal prediction of events (30), it seems likely that it should contribute to appetitive learning and neutral stimulus-stimulus correlational learning as well. Several studies report cerebellar activity in response to a conditioned stimulus associated with an appetitive outcome (57, 90, 91), yet precise role of the cerebellum in this process remains elusive (48, 91). As the neural correlates of appetitive and neutral correlational learning subserved by the cerebellum have not been identified, a comprehensive meta-analysis addressing the specific loci, and subsequently the role, of the cerebellum in this function is warranted.

Our meta-analysis has several potential limitations. First, the included studies differed in design and methodology. More specifically, the studies differed in stimulus types and presentations, timing of the stimuli, conditioning schedules, and procedures. Second, only about half of the studies that fulfilled the criteria reported cerebellar activation during fear learning, which emphasizes the considerable variability in the neuroimaging findings. This could be due to the methodological heterogeneity of the individual studies (33). Third, the meta-analytic approach of the current study provides a quantitative overview of positive results only, and thus should be interpreted with the caveat that it did not take negative findings into account. Lastly, we excluded studies in which a motor or task response was required during the presentation of the conditioned stimuli. However, it is not possible to completely rule out the possibility that cerebellar activation was partially due to more subtle or covert motor processes

such as motor preparation, muscle tension or eye movements related to task performance.

In summary, this ALE meta-analysis revealed that fear learning is associated with activation of several distinct regions of the cerebellum, including the culmen, left and right lobule HIV-V and left lobule HVI, and the cerebellar tonsils. These regions have previously been implicated in fear acquisition, consolidation of fear memories, and expression of conditioned fear responses. Furthermore, the se regions may have a common role in predictive control and temporal processing, tasks that are essential for fear learning in particular, but also associative learning in general. The process of fear learning is thought to be a model for the development and maintenance of anxiety disorders. Previous studies have examined the neurobiology of anxiety disorders by mainly focusing on key structures of the relevant primary circuitry during fear conditioning (92), while omitting secondary regions, such as the cerebellum, and thus precluding our understanding of their role in anxiety disorders. Future studies are needed to clarify the nature and extent of the contribution of the cerebellum to fear conditioning, with the ultimate goal of gaining a more complete insight into the pathophysiology of altered fear learning manifested in anxiety disorders. Finally, the results of the current meta-analysis add to the ever-accumulating evidence that the cerebellum plays a significant role in higher order functions, warranting more attention for the cerebellum and its connectivity in future neuro-imaging studies on cognition and emotion.

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On stress and reward

Chapter 8

Social Stress is Associated with Reward Dysfunction: Converging Evidence from the Laboratory and the Daily-Life

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ABSTRACT

Stress-borne alterations in social functioning confer risk for subsequent development of mental disorders. Reports of stress-induced attenuation of incentive-driven behavior propose a plausible underlying mechanism of this phenomenon, and warrant exploration of the social aspects of the stress-reward connection. In the current study, we investigated the effects of social stress on reward sensitivity by inducing social stress in a laboratory experiment. We then extended this study to real-life by examining the fluctuation in reward experience in relation to exposure to social stress using the experience sampling method. In the laboratory settings, social stress impaired preference for rewards relative to the pre-stress assessment, while sensitivity to punishments remained intact. Simultaneously, exposure to social stress was associated with decreased positive affect and self-esteem. We confirmed these findings in the real world, where social stress was associated with diminished positive affect as well as blunted consummatory and anticipatory reward. These converging results from both experiments add a crucial social dimension to the established depressogenic effects of stress, exposing a link between social stress and compromised hedonic capacity and volition.

INTRODUCTION

Our unpredictable everyday environment exerts its hormetic effect through constant challenges to the status quo. Positive factors, or rewards, drive motivated action, while environmental adversities, or stressors, put a strain on coping resources. Much research has been devoted to the role of impaired ability to respond to the environmental demands in precipitating psychopathology; hyper-reactivity to stress has been shown to act as a potent catalyst in the onset and exacerbation of psychosis (1, 2), PTSD (3) and affective disorders (4)At the same time, a separate line of research implicates aberrant reward function in the motivational deficits accompanying these disorders (5-7). In reality, however, incentives are not encountered in vacuum, but rather experienced against the backdrop of variable degrees of stress, which in turn can warp the perception of the incentive value. Capitalizing on this notion, studies of altered reward experience in the context of stress have recently been receiving increased attention. In experience sampling method (ESM) studies, healthy adolescents and adults were found to derive less pleasure from everyday events following a stressful situation (8). This effect was also demonstrated experimentally, with healthy volunteers responding to threat-of-shock with reduced reward sensitivity (9, 10), blunted consummatory pleasure (11), and impaired reinforcement learning (12). Contrastingly, an enhancing effect of stress (threat-of-shock) on punishment learning has been reported in cognitive behavioral (13, 14) as well as brain imaging studies(15). Taken together, these findings suggest that stress selectively impairs the ability to modulate behavior as a function of rewards.

Within the domain of adverse environmental factors, the noxious effects of social stressors in particular have recently been brought to the fore. Compelling evidence from animal models links social stress to reduced incentive-driven behavior and adaptive exploration of novel environments, while simultaneously accurately replicating the neurobiological signature of anhedonia in human subjects (16). Surprisingly, in the human reward-processing literature, findings specific to social stress are scarce. Using the well-validated Trier Social Stress Task, Plessow and colleagues [35] demonstrated deficits in flexible goal-directed behavior following an aversive social situation. Cavanagh and colleagues (17) reported compromised reinforcement and facilitated punishment learning under social evaluative stress in punishment-sensitive individuals. In all aforementioned studies, the impact of social stress was demonstrated using monetary wins and losses in a highly controlled laboratory environment designed to approximate the stresses and rewards of the everyday life. The generalizability of these tenets to the real world social contexts with their non-monetary incentives is, to this day, only hypothetical. Consequently, the putative effect of social stress on the sensitivity to real world rewards, and by extension the ecological validity of the stress-incentive salience connection, remains elusive.

This article therefore combines two studies; in a laboratory experiment we tested the effects of experimentally induced social stress on reward sensitivity, and in a separate ESM study we investigated the effects of naturally occurring social stress on hedonic experience in the real world.

STUDY I : LABORATORY EXPERIMENT

In the laboratory experiment, we investigated the capacity to modulate one's behavior as a function of incentive before and after the induction of social stress. A probabilistic stimulus selection task (18) previously shown to be sensitive to stress manipulation (12) was modified to deliver social feedback (smiles versus frowns). A widely used ostracism paradigm- Cyberball- was used to induce psychosocial stress in the form of social exclusion(19). In order to investigate the effects of social stress on sensitivity to rewards, and tease out the potentially confounding effects of cognitive load of the stress task, we employed a mixed between-group design: all participants first completed a baseline reward task followed by either a) social stress task (stress group) or b) social control task (control group) after which incentive processing of both groups was re-assessed using an analogous reward task. This design allows to avoid contaminating the reward responsiveness by the distracting properties and cognitive load of the overlaid stressor.

In view of the aforementioned evidence, we hypothesized that compared to control group, participants in the stress group will demonstrate impaired sensitivity to rewards.

METHODS

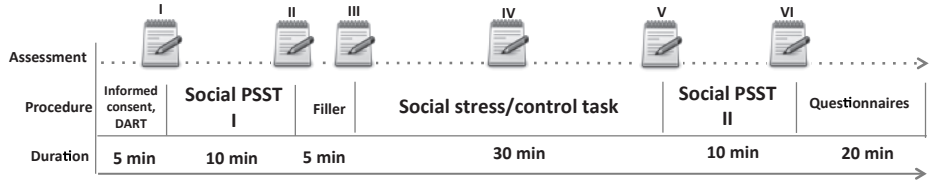
Participants

A total of 47 healthy women were recruited via posters to participate in the study. Of these, 28 participants (M_{age} = 22.3 years) underwent the stress task (stress group), and 19 participants (M_{age} = 21.8 years) completed the control task (control group; $t_{age}(45)$ = 0.58, $p > 0.05$). Due to the recent reports indicating gender differences in reactivity to stress (20)(21) we restricted the study sample to female participants only. All participants were Dutch-speaking students enrolled at the Faculty of Psychology and Neuroscience or in the Faculty of Health, Medicine and Life Sciences of Maastricht University. At the time of the study, all participants denied alcohol, illicit drug, steroid and psychotropic medication use. They were reimbursed for their time and effort with standard coupons worth 20 euros.

Procedures

Upon agreeing to take part in the study, eligible participants underwent a single two-hour testing session. The stress group completed the baseline Probabilistic Stimulus Selection Task (reward task; PSST), a social stress task and the follow-up PSST, while the control group underwent an identical baseline PSST, a control task and an analogous follow-up PSST. In order to ascertain participants' current subjective levels of positive and negative affect, stress and self-esteem, brief psychological state self-reports were purposefully interspersed throughout the testing session (Please refer to figure 1 for the schematic representation of the schedule and duration of all study assessments in the stress and control group.) The session was initiated by obtaining informed consent in accordance with the rules and regulations of the standing ethics committee of Maastricht University. Subsequently, baseline psychological state was established for both groups by asking participants to fill out the first self-assessment, followed by the baseline computerized PSST. Then, another self-report took place and the stress group completed the social stress task, while the control group underwent the control task. Both tasks were interjected by one self-assessment and immediately followed by another self-report. Finally, both groups completed the follow-up PSST and the final self-report. The session was completed upon filling-out demographics and lifestyle questionnaires, after which all participants were debriefed, thanked and reimbursed for their time and effort.

Figure 1: Schematic representation of the schedule of study assessments.



PSST = Probabilistic stimulus selection task. PSST I and II = the two versions of the PSST administered in a counterbalanced fashion. DART= Dutch Adult Reading Test. Min = minutes. Roman numerals denote the order of the momentary self-assessments of social stress, affect and self-esteem.

Probabilistic stimulus selection task

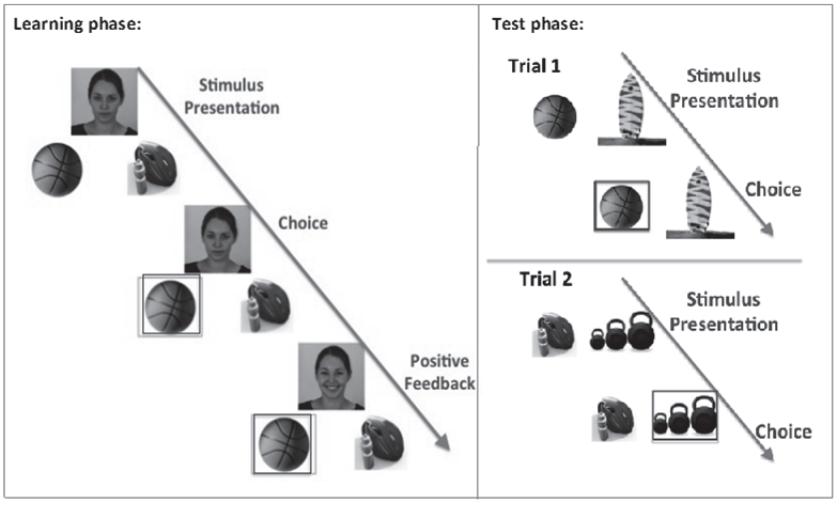
The PSST was administered using E-prime (Psychology Software Tools), presented on a 17-inch laptop computer. One version of the task was completed before and one after the stress induction. The order of the two versions was fully counterbalanced across participants.

The task consisted of a learning phase followed immediately by a test phase (figure 2). In the learning phase, participants were presented with a pair of items displayed side-by-side on the screen, below a color photograph of an actor with a neutral expression. Each actor was associated with a certain pair of items illustrating his or her

hobbies or studies, depending on the version (e.g.: a surf board to illustrate surfing; white coat with a stethoscope illustrating medicine). The participants were instructed to learn what the actors' hobby/study was by choosing one of the items and receiving a feedback: the actor's smile following a correct choice and a frown following an incorrect one. Each actor was associated with a pair of items with different probabilities of reinforcement: 90-10, 80-20 and 70-30. For instance, the choice of the correct item of the 80-20 pair led to a smile on 80% of the trials and to a frown on 20% of trials. Each pair of items was presented 40 times in a random order, for a total of 120 trials.

Immediately upon completing the learning phase, participants proceeded to the test phase in which all previously seen items were presented on the screen in a total of 60 original and novel pairings. On each trial, the participants had to select the item they considered to be the better choice, thus demonstrating their knowledge of the different rewarding properties of the various items. No feedback was provided in this phase. Both versions of the task lasted approximately 10 minutes.

Figure 2: Schematic representation of the probabilistic stimulus selection task



Two versions of the PSST were administered during one testing session, one preceding and one succeeding the stress manipulation. The learning phase of one version contained three actors (2 female, one male) who were randomly assigned two items representing their hobbies. These items were randomly chosen from a pool of 12 items. The learning phase of the other version contained another three actors (2 male, 1 female), each associated with 2 items randomly chosen from a pool of 12 items representing the study of the actors. The test phase of both versions consisted of original and novel pairings of all items without the presentations of accompanying actors or feedback. The order of the two versions was fully counterbalanced across participants.

Social stress task

Social stress was induced using a modified version of the Cyberball paradigm (19). The task began by informing the participants that they would now be joining an online

community of fellow students performing an unrelated experiment. First, they filled out a simple profile by indicating their name, study, hobbies and favourite books and music. Additionally, a picture was taken and uploaded into the profile. Subsequently, participants logged into the game where their picture and profile were displayed on the screen next to their animated character, similarly to the three other players. Participants were instructed to throw an animated ball by clicking on the character of the corresponding player with a mouse. They were informed that they, just as the other players, might freely choose the recipient of their throws. Unbeknownst to them, the other three players were controlled by a computer program in such a way that they threw the ball to the participant a few times, but continued throwing it back-and-forth only among themselves, thus excluding the participant from the game. A total of four 8-minute blocks were completed in this fashion, with each block starting by the participant joining a new group of players.

Following the stress-induction blocks, a brief 4-minute stress reversal condition was administered to ensure that participants' affect, self-esteem and perception of social stress returned to baseline levels before proceeding to the reward task. The stress reversal phase was identical to the stress induction phase in that participants joined a new group of players in the same virtual environment as before, and initially received equal number of balls as before. However, as the block progressed they received increasingly more balls until becoming the most popular player in the group by the end of the block.

Control task

The control task was designed to retain the social character, cognitive demands and design of the stress tasks, without inducing any social stress. In this condition, participants entered a virtual environment similar to that of the social stress task (detailed in the previous subsection) where they were throwing an animated ball with three other players by clicking on their picture with a mouse. Instead of freely choosing whom to throw the ball, however, a changing colourful shape (circle, triangle or square) visible to all players at all times dictated the recipient of the next throw. By introducing a bounding rule, the possibility to exclude or favour any of the players was eliminated, and all players received an equal number of throws. Furthermore, participants entered the game as an anonymous players, without any personal profile information nor picture being displayed on the screen for the other players to see.

The stress group also completed 5-minutes of this control condition following the baseline PSST to ensure that any potential change in dispositional state that may have been caused by the PSST did not influence the effectiveness of the upcoming stress manipulation.

Momentary self-assessments

A total of six in-the-moment self-reports were completed throughout the single study session at a priori chosen time-points of interest (please refer to figure1 for the schedule and timing of the assessments). Each questionnaire contained 22 items rated on a 7-point Likert scale (ranging from 1 = *not at all* to 7 = *very*). At each time-point, these ratings provided subjective appraisals of the current situation, company, mood, self-esteem, as well as the extent of perceived social exclusion and stress.

Statistical Analyses

Momentary self-assessments

To track the fluctuations in dispositional states, four main constructs were devised using the self-reports: positive affect (PA), negative affect (NA), self-esteem (SE) and social stress (SS). Factor analyses, performed in STATA 11 (StataCorp. 2009), revealed that introspective items 'I feel… cheerful','relaxed' 'content', and 'enthusiastic' loaded on the PA factor (Cronbach's alpha = 0.75); whereas items 'I feel..'irritated', 'anxious' and 'sad' loaded on the NA factor (Cronbach's alpha = 0.71). The items "I doubt myself" and the reverse coded "I like myself" clustered around the SE factor (Cronbach's alpha = 0.86). The social context items 'In this company I feel… 'inhibited', 'appreciated'(reversed), 'connected' (reversed), 'comfortable' (reversed) and 'this is a pleasant company' (reversed) formed the SS factor (Cronbach's alpha = 0.86). The composite scores of all four constructs were computed at each time point. First, in order to compare the two groups on the subjective SS, PA, NA and SE experienced during the stress/control task, a difference score was computed for each participant by averaging the two scores obtained during the stress/control task, and subtracting the score assessed before the task from this number. The difference score was then entered into a simple regression analysis as the outcome variable.

Reward and punishment learning

The learning phase of both the pre-stress and post-stress reward task was divided into eight blocks of five trials per pair, and the percentage of correct choices (choices of the more frequently rewarded stimulus) of each pair was calculated. To compare the two groups on reward learning in the PSST at baseline and follow-up, a multilevel mixed regression was performed for each pair (90:10, 80:20, 70:30), with two levels per individual (two observations: baseline, follow-up). In this equation, percentage of correct choices of the pair constituted the dependent variable (DV), and group (stress, control), time (baseline, follow-up) and the interaction between group and time were entered as the predictors. The structure of the matrix was set to covariance (unstruc-

tured), since observations within each individual are expected to co-vary, meaning that each individual is expected to perform similarly at baseline and follow-up.

Social reward and punishment sensitivity

For the purposes of this experiment, 'sensitivity' reflects the capacity to transfer the incentive value of each item from the learning phase into the test phase. More specifically, reward sensitivity was defined as the preference for the most advantageous item -the item previously most frequently associated with rewards - over its less rewarding alternatives, when these appeared in *novel* contexts. Punishment sensitivity was conceptualized as the avoidance of the most frequently punished stimulus in favour of its relatively better counterparts, when presented in *novel* pairings. To this end, a new 'choose best' variable was created by computing the percentage of choices of the 90 item in all instances in which it was newly paired with the relatively worse items (80, 70, 30, 20). Analogously, the 'avoid worst' item was created by computing the percentage of choices of the relatively better items (30, 20, 80, 70), when these were newly presented with the worst 10 item.

This procedure was performed for the baseline test phase as well as for the follow-up test phase. To compare the two groups on their incentive-driven performance at baseline versus follow-up, two multilevel mixed regressions were carried out, one with percentage of correct choices of the best stimulus as the DV and another one with the percentage of correct avoidance of the worst stimulus as the DV. In both analyses, group (stress, control), time (baseline, follow-up) and the interaction between group and time were entered as predictors. The structure of the matrix was again set to covariance(unstructured).

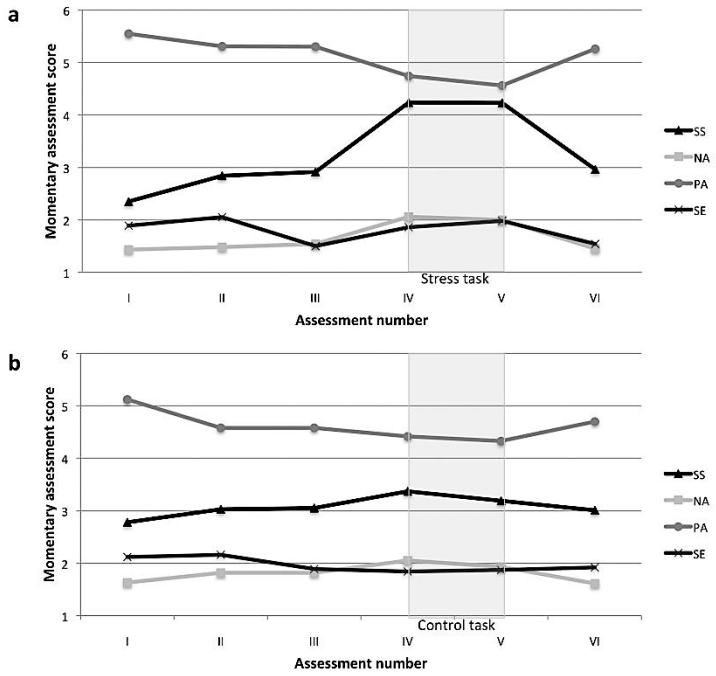
RESULTS

Manipulation check: momentary self-assessments

A series of regression analyses (yielding regression coefficient β) was conducted to compare the stress and control group on the change in levels of momentary social stress, affect and self-esteem during the stress/control task. As expected, the two groups differed significantly in the extent to which SS, PA and SE changed during their respective tasks relative to the pre-task assessment, with the stress group reporting significantly greater increase in SS and decrease in PA and SE than the control group. However, increase in NA in the stress group was only numerically greater than in the control group, and did not reach statistical significance (graph 1 and table 1).

Importantly, both groups endorsed comparable levels of SS, NA, PA, and SE at the initiation of the first and the follow-up reward task, immediately after the completion of both tasks, and after debriefing at the end of the testing session (all $p > .05$, graph 1).

Graph 1: Momentary self-assessments of social stress, affect and self-esteem



Fluctuations in subjective social stress (SS), positive affect (PA), negative affect (NA) and self-esteem (SE) throughout the testing session. All items were rated on a 7-point Likert scale ranging from 1=not at all to 7= very much. Assessment I = before baseline social reward task (PSST), II = after social reward task, III = before social stress/ control task, IV = during social stress/control task, V= immediately after social stress/control task, VI = after social reward task (PSST). Grey area indicates the administration of social stress/control task. (a) The social stress task was associated with increase in subjective SS and decrease in PA, NA and SE. (b) subjective mental state remained unchanged in the group exposed to the control task.

Table 1: Changes in momentary mental state associated with social stress and control task

Momentary mental state	Stress Group		Control Group		Group Difference	
	M	SD	M	SD	β	p-value
Δ SS	1.32	0.82	0.23	0.57	1.09	0.000
Δ PA	-0.85	0.81	-0.20	0.45	-0.45	0.035
Δ NA	0.49	0.64	0.17	0.72	0.33	0.111
Δ SE	-0.42	0.71	-0.04	0.66	0.46	0.031

Mean changes (Δ) in momentary social stress (SS), positive affect (PA), negative affect (NA) and self-esteem (SE) endorsed by the group exposed to the social stress (N=28) and controls (N=19), and the difference between the two groups. Δ = mean of the scores endorsed during and immediately after the stress/control task minus the score from the pre-stress/control task assessment. Results of the mixed regression analyses show significantly greater increase in subjective SS and decrease in PA and SE in the stress group compared to the control group.

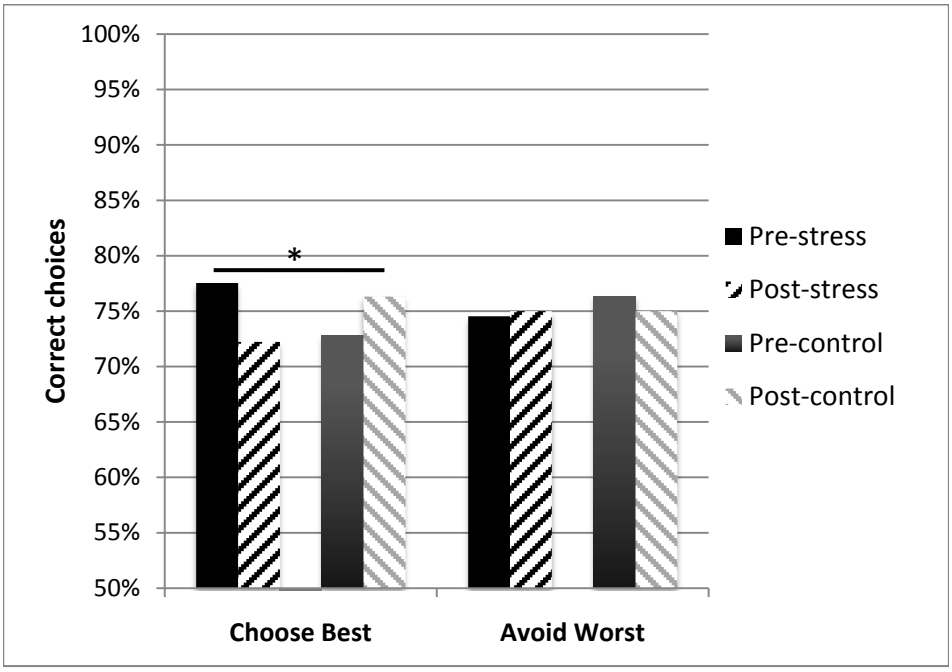
Reward and punishment learning

All participants achieved successful learning of the contingencies by performing above chance of all three pairs in both the pre- and post-stress learning phase. Importantly, the mixed multilevel regression yielded no significant effect of group X time on the accuracy on the 90-10 pair (*B* = -.06; *SE* = .08, *p* = .406), 80-20 pair (*B* = .02; *SE* = .08, *p* = .835) and 70-30 pair (*B* = .11; *SE* = .17, *p* = .544), indicating no effect of stress on acquisition of the initial contingencies.

Reward and punishment sensitivity

Multilevel mixed regressions were performed to detect the effect of group (stress/control), time (baseline/ follow-up) and the interaction of the two on the accuracy on the *novel* reward-driven ('choose best') and punishment-based ('avoid worst') performance in test phase. As evidenced by graph 2, a significant group x time emerged on reward-driven responding (*B* = -.19; *SE* = .07, *p* = .008), with the stress group's accuracy on the 'choose best' deteriorating post-stress (*M* = 71.9, *SD* = 14.7) compared to pre-stress assessment (*M* = 79.5, *SD* = 19.1), and the control group's performance improving somewhat from pre-task (*M* = 72.8, *SD*= 25.2) to post-task assessment (*M* = 75.3, *SD* = 21.1). Contrastingly, using the multilevel mixed regression, no group x time interaction was detected on the accuracy on the 'avoid worst' item (*B* = -.04, *SE* = .07, *p* = .579), indicating that avoidance-based behaviour remained intact after stress (graph 2).

Graph 2: Effect of social stress on incentive-based responding in a probabilistic social reward task



Overview of accuracy (percentage correct choices) in the choose reward and avoid loss domains of the acquisition and test phase of the PSST pre- and post-stress/control task. Choose Best = percentage choices of the 90 item when presented in novel pairings with the 80, 70, 30 and 20 items. Avoid Worst = percentage choices of the 80, 70, 30 and 20 items when appearing in novel pairings with the 10 item. Pre-stress = accuracy on the baseline PSST prior to exposure to the social stress task (N=28) Post-stress = accuracy on the follow-up PSST after exposure to the social stress task (N=28). Pre-control = accuracy on the baseline PSST prior to exposure to the social control task (N=19). Post-control = accuracy on the follow-up PSST assessed after exposure to the social control task (N=19).

* = the difference between the two groups ($p < 0.05$) in PSST accuracy at follow-up relative to baseline.

STUDY II: EXPERIENCE SAMPLING IN THE DAILY LIFE

The intriguing finding of deleterious effect of social stress on reward sensitivity evident in the laboratory study prompted the investigation of this phenomenon in the naturalistic settings of the everyday life. In the ESM study, 17 healthy volunteers appraised their current mood and context multiple times a day for the duration of one week. To ensure comparability between the laboratory and the ESM study, the items assessing momentary social stress and affect overlapped in both studies to the extent possible. Consequently, the tenets of the experimental study - changes in mental states and reward responsiveness as a function of social stress- could be examined in the flow of daily life. Since the main aim of the ESM study was to investigate whether the depres-

sogenic effects of social stress detected using a highly controlled and standardized experiment generalize to the complex circumstances of the everyday life, a number of constraints were relaxed in the ESM study: i) both male and female participants were included in the sample, and ii) affect and reward experience were assessed concurrently with social stress.

METHODS

Participants

A total of 17 healthy individuals (six males, M_{age} = 38.34 years) were recruited via advertisements to participate in the study. The inclusion criteria were age between 18 and 65 years and sufficient command of the Dutch language to understand the questionnaires. Exclusion criteria consisted of organic brain disease, history of head trauma with loss of consciousness, current or lifetime Axis I or II diagnosis and current or lifetime illicit drug and alcohol abuse or dependence.

Procedures

The experience sampling method (22, 23) was used to conduct momentary self-assessments on six consecutive days. Participants were provided with a digital portable device, psymate®, programmed to emit 10 signals ("beeps") at unpredictable moments between 7:30 and 22:30, on average once every 90 minutes. Every beep was a prompt to fill out a brief questionnaire that appeared on the screen. The study used a semi-random beep schedule with the constraint that the beeps could not occur within 15 minutes of each other, ensuring that every time of the day had equal probability of being sampled for assessment. Participants were instructed to fill out the questionnaire immediately after each beep, and extensively trained on how to do so. Each questionnaire consisted of questions on the current affect, behaviour and context rated on a 7-point Likert scale ranging from 1=*not at all* to 7=*very much*. In order to minimize memory bias, each questionnaire was only available for 15 minutes after the beep. Throughout the six days of the ESM sampling period, all participants were called to ensure understanding and compliance with the instructions, and a researcher was available by a telephone at all times in case of questions or problems.

Measurements

Momentary appraisal of social stress

Social stress (SS) was assessed using five items rated on a 7-point Likert scale (from 'not at all' (1) to 'very much' (7)): 'In this company I feel… 'inhibited', 'judged', 'accepted' (reversed), 'connected' (reversed) and 'this is a pleasant company' (reversed). These items were chosen to overlap with the momentary assessment items from Study I with the exception of the item 'judged' that has previously been shown to be suitable for assessments in the daily life, but in the laboratory study could be interpreted as the feeling of being judged by the experimenter who observes the session. The selected items had high internal consistency (Cronbach's alpha= .77), comparable with the SS items in Study I.

Momentary appraisal of affect

Positive affect (PA) was assessed using four adjectives rated on a 7-point likert scale: 'I feel… cheerful', 'content', 'relaxed' and 'enthusiastic', while negative affect (NA) comprised of items 'I feel… irritated', 'sad', and 'anxious'. Factor analyses were carried out to confirm that the selected items loaded on the latent PA and NA factors, respectively. The items had good internal consistency (Cronbach's alpha for PA = .81 and NA=.66) and overlapped entirely with the PA and NA items from Study I.

Momentary appraisal of pleasant events

The pleasantness of a recent event (PE_r) was assessed by instructing the participants to think about the most important event since the last beep, and to rate how pleasant it was on a bipolar scale ranging from very unpleasant (-3) to very pleasant (+3), with 0 being neutral. Only the positive part of the scale (0 to +3) was taken into account during analyses.

Similarly, the anticipation of pleasantness of a future event (PE_f) was assessed by instructing the participants to think about the most important event in the upcoming hour, and to rate how pleasant they expect it to be on a bipolar scale ranging from very unpleasant (-3) to very pleasant (+3), with 0 being neutral. Again, only the positive part of the scale (0 to +3) was used in analyses.

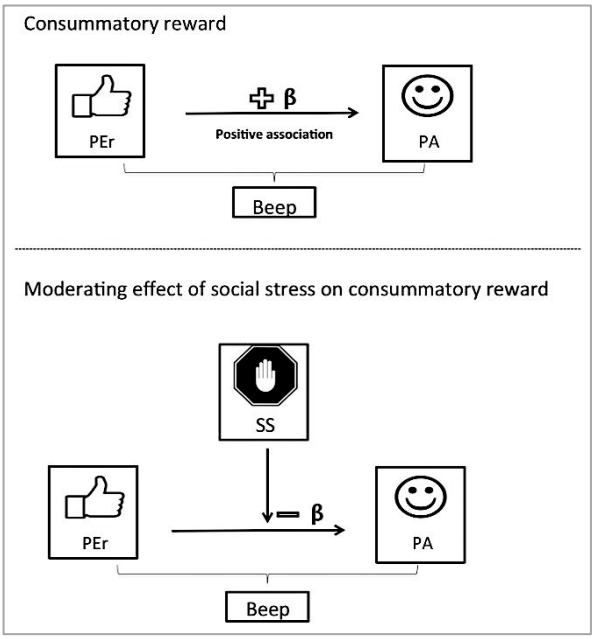
Analyses

All analyses were conducted in STATA 11.0 (StataCorp, 2009) using multilevel mixed regression which is suitable for ESM studies due to the hierarchical character of the data: within-subject momentary ratings at every beep (level 1) are nested within individuals (level 2). First, we operationally defined consummatory reward as the extent to which recent pleasant events generate positive affect. The strength of the association

between PA and PE_r across the whole sample was assessed with a multilevel regression analysis with PA as the outcome variable and PE_r as the predictor (Figure 3). Anticipatory reward was operationalized as the expectation of pleasant events in the near future (upcoming hour; PE_f).

In order to test whether social stress was associated with changes in consummatory reward, a multilevel regression analyses was conducted with PA as the outcome variable and the PE_r, SS, and their interaction as the predictors (Figure 3). Stress-related changes in anticipatory reward were tested by performing a multilevel regression analysis with PE_f as the outcome variable and SS as the predictor. All analyses were corrected for age and gender.

Figure 3: Schematic representation of the operational definition of consummatory reward and the impact of social stress on consummatory reward



At every beep, pleasantness of a recent event (PE_r), positive affect (PA) and social stress (SS) were assessed by the respective items on the ESM questionnaire. Consummatory reward was defined as the association between PE_r and PA, represented by a β coefficient with a positive sign. SS was found to be a moderator of this relationship.

RESULTS

Consummatory reward

First, the multilevel regression analysis confirmed main effect of pleasantness of a recent event on positive affect (*B*=.25, *SE*=.03 *p*=.000), indicating that increasing event pleasantness was associated with higher PA. Additionally, a main effect of social stress on affect emerged, with higher social stress being associated with lower PA (*B*=-.34, *SE*= .05 *p*=.000) and higher NA (*B*=.29, *SE*=.04 *p*=.000). Importantly, a significant SS x PE_r interaction emerged (*B*=-.12, *SE*=.04 *p*=.004), indicating that social stress moderates the association between PE_r and PA, in that higher SS is associated with lower consummatory reward.

Anticipatory reward

The multilevel regression analysis revealed significant negative association between SS and PE_f (*B*=-.44, *SE*= .07 *p*=.000), indicating that anticipatory reward decreases with increasing social stress.

DISCUSSION

The current study assessed the effect of social stress on sensitivity to rewards using two approaches; in a laboratory experiment, we tested the capacity to modulate behavior as a function of rewards before and after the exposure to social stress versus control condition. In a daily-life ESM study, we investigated the moment-to-moment fluctuations in subjective reward experience in relation to varying degrees of social stress. The results of both studies converged on the deleterious effects of social stress on reward sensitivity and experience.

Experimentally-induced social stress

Consistent with our hypothesis, in the laboratory experiment significant attenuation in reward sensitivity emerged following social stress relative to the pre-stress assessment, whereas preference for rewards slightly improved following the non-stressful control condition. Simultaneously, the tendency to avoid punishments was found to be unaffected by both exposure to social stress and control condition. As expected, the induction of social stress was also accompanied by self-reports of significantly higher subjective social stress and lower positive affect and self-esteem, while no changes in subjective mental states were endorsed by the control group. Stress-bound increase in negative affect detected at a descriptive level did not reach statistical significance.

These results align with nearly uniform reports of the impact of stress on reward dysfunction. Furthermore, stress-induced impairments in reward processing similar to those observed in the present study have reliably been detected using a variety of tasks including the classical MID (24), signal detection (11) and PSS task (25) administered both concurrently (10) and consecutively (8) with the stressor. The novelty of the present study lies in the finding that social exclusion can elicit reward dysfunction akin to the effects induced by electroshocks and negative evaluations employed previously.

Daily-life social stress

These intriguing results prompted the inquiry into the impact of daily life social stress on consummatory and anticipatory reward on a moment-to-moment basis. The ESM study tied in with the laboratory experiment by showing that consummatory reward conceptualized as the extent to which positive affect rises in response to recent pleasant event was moderated by the degree of social stress. In turn, anticipatory reward, defined as the capacity to look forward to pleasant events in the upcoming hour, was inversely related to the current social stress.

These findings confirm the results from our laboratory experiment and provide ecological validity to the observed depressogenic effects of social stress. Furthermore, they suggest that social stress may be added to the array of naturalistic stressors eliciting reward dysfunction, which to day ranged from minor hassles (8) to military service in high conflict areas (26). In the latter study, reward sensitivity of young healthy soldiers was assessed before and after active duty in combat areas. Brain imaging revealed attenuated responsiveness to rewards following exposure to stress in nucleus accumbens, a key reward-signaling area. Importantly, stress-related blunting of reward sensitivity was most pronounced in individuals endorsing higher depressive symptoms after deployment (26). The present study contributed a novel social dimension to the naturalistic stressors, highlighting the substantial depressogenic potential of acute social stress in the everyday life.

It stands to reason that the deleterious effect of social stress might be an adaptive response rooted in our evolutionary history; individuals for whom social stresses was emotionally relevant enough to guide behavior might have been most likely to collaborate with other humans on survival. Our inherent need for belongingness and aversion to social discord is reflected in the modern society's use of social punishments, ranging in severity from criticism to isolation cells in prisons. The current article demonstrates the mechanism of adaptive emotional and behavioral reactivity to social stress on the micro-level of the human experience, opening avenues for research into the putative role of abnormal reactivity to social stress in the precipitation of psychopathology.

There are several notable limitations of the laboratory experiment that we attempted to mitigate by performing the experience sampling study. First, due to sex differences in reactivity to stress (20), the sample selected for the laboratory experiment consisted entirely of women. In this regard, it is important to acknowledge the

relatively higher inherent and acquired sensitivity to social cues among women, which could prime their behavioral and psychological reactivity to the social stress and feedback (27) (21).While this notion likely affected the results of the experimental study, we tested the generalizability of its findings to both men and women participating in the ESM study, and observed similar affective and reward reactivity to social stress across the entire group. Second, in order to approximate naturalistic settings, we opted for a within-subjects repeated measures design in which incentive sensitivity was determined before and after the induction of social stress. This procedure could potentially introduce the test-retest effect and lead to improved performance at post-test. Although we partially mitigated this problem by administering two versions of the PSST that were fully counterbalanced across participants, we were not able to control for growing experience with the task. The fact that participants showed *reduced* accuracy in the reward domain despite their advantage at retest substantiates the robustness of our findings. Third, following the stress manipulation, a brief stress reversal condition was introduced in order to prevent stress-induced negative affective state from contaminating the subsequent incentive processing. Therefore, we sacrificed ecological validity in favor of being able to i) equate the affective states at pre and post-test for more precise comparisons and ii) disentangle the effects of stress-bound affective negativity from the effects of stress itself. The experience sampling study confirmed, however, that the findings acquired in such strictly controlled conditions do, in fact, translate to the complex circumstances of the everyday life.

Collectively, evidence from clinical (28, 29) studies unequivocally establishes the intertwined relationship between stress and reward, with subjective reactivity frequently appearing at its intersection. The rationale for the present studies was derived from two main lines of evidence: psychosocial stress is believed to be the main source of adverse experiences, and may play important role in the etiopathogenesis of disorders related to reward dysfunction such as addiction, depression and psychosis (11, 30); compromised social functioning manifested through social anhedonia and avolition often accompanies psychopathology (31, 32), thus interfering with the formation of adequate social support required to cope with stressors (33). We examined the plausibility of the first part of this maladaptive cycle, and show noxious effects of social stress on the sensitivity to rewards

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Chapter 9

General Discussion

This dissertation aimed to reveal a section of the nuanced and extraordinarily complex mechanism of responding to the environment that in the brain imaging lingo would be termed axial. This, because akin to the brain, the environmental reactivity was presented from three different angles: behavioral, affective and neurochemical. Additionally, in all forms of responsiveness to the stresses and rewards, in both psychosis and health, the brain was found to play a pivotal role. It is therefore tempting to credit the brain with a causative one, but this might not be entirely justifiable.

Brain: presumption of innocence

The anticipation, reception and learning from rewards was found to elicit significant increase of dopamine activity in the mesolimbic parts of the brain of healthy individuals with and without a genetic predisposition to psychosis (chapter 2). Moreover, the more extensive the DA activity in the ventral striatum, a key portion of this region, the more advantageously the participants behaved in the reward task. These findings confirm that reward responsiveness is modulated by striatal DA activity, and that as its size increases, so does reward sensitivity. The findings of unaltered and highly specialized reward-induced DA activity in the striatum of healthy relatives at-risk for psychosis deserve special attention. Considering that these individuals share the patients' genetic predisposition, yet they remained healthy, enjoy high quality of life (1) and adequate reward function (2, 3), one could reasonably speculate that preserved DAergic modulation of reward responsiveness might mark robustness against the disease. Corroborating evidence from functional magnetic resonance imaging (fMRI) relying on blood flow to the brain regions as an indicator of their activity, confirms that healthy first-degree relatives of patients demonstrate normal (2-4) and even supranormal neural striatal activity to reward feedback (5). In conclusion, optimal DAergic and neural activity to rewarding outcomes in the striatal regions of healthy relatives likely provides the reward teaching signal that drives incentive-based choices, a mechanism that might foster resilience to the aberrant salience associated with the descent into psychotic illness(6). This intriguing and promising finding warrants replication in this population, and corroboration in samples further on the spectrum of psychosis.

The behavioral study of reward responsiveness in chronic patients echoes the notion that reward deficit is a key component of maladaptive behavior in full-blown psychosis (chapter 3). Although patients learned from rewards just as well as healthy controls, those with the most severe negative and positive symptoms performed paradoxically best. This was, however, due to choosing a highly unusual strategy, likely attributable to the lack of flexibility and exploration (7), again pointing towards an aberrant modulation of behavior based on its outcome in the group with the greatest aberrant salience.

Meanwhile, acute psychosocial stress was found to induce an increase in DA activity in the prefrontal and temporal cortices of healthy individuals, with a comparable

effect on patients with psychosis (chapter 5). A difference between the groups emerged, however, only when another factor was taken into account- early life stress (chapter 6). While in controls higher childhood trauma was asoociated with a potentiated DAergic stress response in the medial prefrontal cortex, no such pattern was found in patients, pointing towards deviant calibration of stress-mediating mechanisms in this group. It is important to emphasize, though, that despite this lack of putative adptive mechanism in the patients, as a group, they demonstrated appropriate prefrontal DAergic reactivity to acute stress, as evidenced in chapter 5.

The case against environment

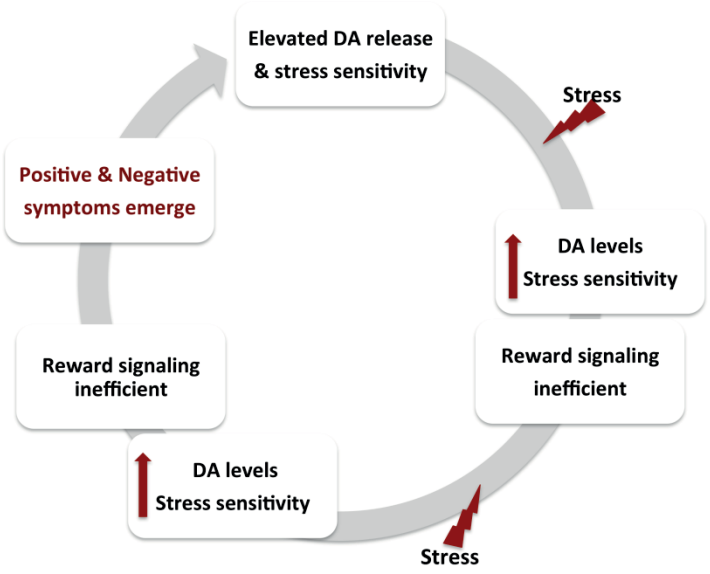
Collectively, when both of the neurochemical studies of DAergic reactivity to reward and stress are considered in isolation, they prove largely inept in discerning between healthy individuals and those on the psychosis continuum. This contradicts the dopamine hypothesis of psychosis, the notion that the faulty brain with its dysregulated DA system drives the aberrant salience associated with abnormal behavior, and ultimately, psychosis (8). Moreover, even though this assumption seems to be supported by the observation that the dopamine blockade by antipsychotics dampens the positive symptoms (9), the success of the pharmacological therapy by itself has, likewise, been limited (10). It might thus be reasonable to challenge the causality of the relationship between DA and psychosis, and subject it to a rigorous scientific scrutiny, especially considering the fact that the same reasonig that arrives at the dopamine hypothesis, could apply to the common flu: since there are higher levels of inflammatory cytokines in the lung tissue of those infected with the influenza virus (11), impaird lung reactivity to the virus appears to be linked to the illness. Moreover, since Aspirin has been found to relieve the symptoms of the flu and decrease the inflammation levels in the lungs (12), the inflammation is likely the final common pathway to this lung disorder. This assumption is, of course, false, because it is known that the increased inflammation is the adaptive reaction to the flu, which is in reality caused by the interaction between the influenza virus and a compromised immune system (11). Analogously, one could entertain the possibility that psychosis is no more caused by DA excess than the flu is caused by Aspirin deficiency. Instead, it might be conceivable that altered DA tone in psychosis, similar to the increased inflammation during the flu, is an appropriate response of the body to an adverse external trigger, in this case stress. In this scenario, dopamine constitutes the stage on which psychosis unfolds, but the environmental stressors likely direct the play.

The findings from the studies in healthy controls support this view (chapter 8). Stress was shown to selectively decrease the sensitivity to rewards, possibly via an acute increase in the DA tone in the mPFC (chapter 5) and striatum (13), putatively precluding the phasic bursts to rewards to reach the receptors (6). This interpretaion is corroborated by the finding that sensitivity to punishments, believed to be propagated

through dips in DA firing (14), appeared to be spared under stress (chapter 8). Simply put, in a striatum that is briefly "flooded" by stress-induced DA, its sudden drop likely has a higher chance of being registered than a boost. Curiously, this exact pattern of preserved sensitivity to punishments in the presence of impaired reactivity to rewards has repeatedly been demonstrated in psychosis, and related to the severity of the negative symptoms (7, 15). It is therefore plausible that the transient decrease in sensitivity to rewards observed in healthy people under acute stress could serve as a model for the perpetual state of reward deficit in psychosis.

The findings from chapter 4 support this view by showing that the capacity to experience pleasant activities as such is an imperative prerequisite for reward learning in the daily life: the experience of positive affect contingent upon a certain behavior, in this case being in the company of others, increased the odds of seeking the company of others in the near future. It is reasonable to assume that the noxious effect of social stress on reward sensitivity demonstrated in the daily life (chapter 8) would interfere with this adaptive cascade. If a mere simulation of a short, mild social exclusion, or a slightly unpleasant company are potent enough to dampen reward sensitivity, and by extension probably also reward learning, what effect could discrimination and economical inequality, the realities of our modern society, have? Profound, according to research, and psychosis might be a prime example. Social defeat brought upon by racial, socioeconomical and physical disparity has been strongly linked to psychotic illness (16, 17), and a recent report confirmed that indeed, minority status even independent of psychosis was associated with striatal DAergic excess (18). Consequently, natural variation in DA synthesis capacity combined with chronic exposures to adversities could, over time, set off a vicious cycle: hyperdopaminergia in the striatum giving rise to acquired hypersensitivity to stress and hypo-reactivity to rewards. These effects then become further potentiated with each additional stressor until they reach critical mass, and aberrant salience emerges. Dopamine is, then, similarly to the inflammatory cytokines in the flu, a mere attempt at self-regulation of the brain under environmental attack.

Figure 1: Illustration of the vicious cycle of dopamine sensitization



This simplified model illustrates the cycle of aberrant reactivity to stress and reward that begins by naturally elevated DA release in the striatum, the neural substrate of increased stress sensitivity. Upon exposure to stress, DA levels further increase and with them stress sensitivity, which creates noise that impairs the propagation of the reward signal, resulting in compromised neural and behavioral reactivity to rewards. These effects are further potentiated with each stressor that has greater and greater impact on the system, until it reaches the point when positive and negative symptoms of psychosis emerge.

Environment: guilty as charged?

This is a subtle distinction from the dopamine hypothesis of psychosis, but its implications for treatment strategies could be far-reaching. To better illustrate this point, consider an example from non-human animals; deers are often mocked for their tendency to freeze when startled by an approaching car, especially at night. In fact, the expression "deer in the headlights" describes the very moment of petrified inability to respond promptly to a frightening stimulus, and is used jokingly, because the deer's reaction is comicly inadequate. This might not be fair, however, since in evolutionary terms, cars are a very recent invention, and the deers simply did not have the time to adapt their reactions. When confronted with a car, they do the very best thing their biology evolved to do in uncertainty, some more so than others. Why would it be so different for humans suddenly facing massive overcrowding and urbanization?

The concept of gene x environment interactions on the dopaminergic dysregulation in psychosis is not new (19). Countless studies have been conducted to parse out the exact environmental triggers (20), the genes mediating their effect (21), and the precise direction and localization of their interplay on dopamine signaling (22, 23). After

all, the distinction between dopamine being the cause or effect of aberrant environmental reactivity is particularly meaningful when designing rational treatments for psychosis. While informative and valid, however, this reductionist approach to intervention might have reached a point of diminishing returns, again illustrated by the deer in the headlights problem.

To closely examine the dysfunctional interaction between the deers and the cars, much research could be devoted to specifying the exact biological markers that make some of the deer more susceptible to the freeze reaction than others. Once that is known, the most frightening properties of the cars would be investigated: the light spectrum, sound frequency, speed at which it is approaching and their mutual relationship. Consequently, if it is decided that some features of the cars are solely to blame, one could abolish them, but that is unrealistic in this day and age. Conversely, if it becomes clear that the biology of the deers makes them ill-equipped to cope with the cars, medicating the deers to normalize this deficit would be sensible, but since that only addressess one part of the issue, it could eventually prove insufficient. Only when the paradigm shifts towards the understanding that the entire interaction is broken, the most logical next step would be to accept that lethal cars and the frightened deers will continue encountering each other, and teach the deer an extensive, functional repertoir of responses to this situation- improve its reactivity. Translated to individuals manifesting aberrant interactions with the inevitable environmental adversities, this might include synergistic lifestyle interventions: stress-management techniques together with sleep hygiene, diet optimization, social interconnectedness and abundance of movement, ideally in nature. At the very least, we might be inclined to question whether it is still justifiable to define mental health as the state of being well adjusted to an unhealthy environment.

A future without a precedent

A critical first step in this direction would be to add meanings to the physiological findings by connecting them to the behavior, affect and symptoms that make the true difference between mental health and disorder. There is thus a need to synthesize measures of neural and chemical responses to computer-simulated events, with the self-reports of in-the-moment affective and behavioral reactions to real-world events. In the realm of environmental reactivity to streses and rewards, the real-life manifestation of (a)motivation, (a)sociality and (an)hedonia could therefore be studied in conjunction with their neural and neurochemical underpinnings. The integration of PET, fMRI and experimental accounts of environmental reactivity with ambulatory assessments such as ESM, heart rate variability, calorimeter and actimeter would provide a comprehensive and unbiased account of the neurobiological bases and real-life consequences of reward-oriented behavior.

The next step would be to actively supplement the environment in shaping the goal-oriented behavior, without any further need to monitor the brain. The data gathered through the periferic self-quantification (subjective and objective ambulatory assessments) would provide integrated real-time, real-world feedback on the current functioning, prompting the change of the status quo, no matter how subtle. For instance, negative feedback informing about the activity, heart rate variability and mood dropping significantly below the cummulative average might, at first by chance, change the current behavior: a person might not have the possibility to get out of a traffic jam, but could try improving posture, performing a breathing exercise to relieve some tension, and thinking about an upcoming vacation instead of the incompetence of the surrounding drivers. The next positive feedback reflecting improvements in mood and physiological arousal would solidify the positive behavioral change for this particular driver, and increase the chances of engaging in a similar stress-managing technique in the future. The ultimate goal is to, over time, autonomously recognize the negative internal and external environment and possess the behavioral repertoire to self-regulate the reactivity to it. Surely, the brain functions best when the person behaves in a way he or she evolved to: not fighting and evading the present environment, but effortesly interacting with it.

This dissertation aimed to begin to elucidate how the environmental cues impact our behavior, affect and neurochemistry, and through what nuanced mechanisms. Expanding this understanding could eventually lead to the development of effective and practical behavioral changes that would optimize the interactions with the environment. The next, and perhaps the most challenging endeavor, would be to design a tool that would assist with the implementation of these strategies, so that reward-driven, goal-oriented behavior becomes the new default in psychosis and beyond.

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Summary

Our goal in any given environment is to gain rewards, avoid losses, and mitigate stressors. In fact, successful pursuit of our desires despite the pressures of the everyday life is an essential marker of mental health. Conversely, reduced capacity to cope with stressors and engage in motivated behavior is the hallmark of severe psychiatric diagnoses, most notably the psychotic disorder. This dissertation explores the many facets of the healthy as well as compromised environmental reactivity in relation to the symptomatology of psychosis. The first section, *On reward,* elucidates mechanisms of reward sensitivity, the second section termed *On stress* focuses on stress responsiveness, and the last section, *On stress and reward*, ties both phenomena together by exploring the effect of stress on the capacity to experience reward as such.

C**hapter 1** introduces the current state-of-the-art in research into reactivity to stress and reward in healthy state and psychosis. Reward capacity is typically tested using computerized tasks in which incentives are used to guide behavior, while stress is experimentally induced using social evaluation and exclusion. This way, both reward and stress processing is shown to engage the striatal and prefrontal regions of the brain, and thought to depend on dopamine signaling. Individuals with psychosis demonstrate robust insensitivity to rewards linked to severe motivational impairments, in the presence of hypersensitivity to stress accompanied by psychotic symptoms. There are critical gaps in our understanding of the neurochemical, behavioral and affective correlates of this abnormality, precluding the design of rational interventions.

On reward

Chapter 2 presents an account of neurochemical modulation of reward processing in the striatal and limbic areas of the brain in individuals with a familial predisposition to psychosis (healthy first-degree relatives of patients) and healthy controls. Positron emission tomography with the ligand 18F-fallypride indexing dopaminergic transmission detected unaltered reward-induced dopamine activity in the predisposed group, possibly conferring resilience to the disorder. Additionally, this study revealed that greater extent of dopamine activity during reward processing in the most of striatum is associated with better performance on the reward task, highlighting the specific role of the striatum in reward-oriented behavior, and preservation of this specificity in individuals predisposed to psychosis.

Chapter 3 investigates behavioral reactivity to rewards and reveals that patients with the most severe symptoms of psychosis adopt, paradoxically, the most advantageous strategy in a reward task. Upon closer look, however, this behavior appears to represent perseveration and inflexibility rather than true supra-normal reward sensitivity, also reflected in the clinical profile of pronounced motivational impairments in this group of patients.

Chapter 4 presents the link between reward deficits and avolition by identifying affective experience of reward as the driving force of motivated action in the everyday life. The experience sampling method applied to a substantial sample of healthy participants reveals that positive affect experienced while performing an activity significantly increases the odds of engaging in that activity in the near future, thus identifying optimal reward sensitivity as the imperative prerequisite of the propagation of reward-oriented behavior. Translated to psychosis, attenuated responsiveness to rewards demonstrated by the patients could potentially hinder goal-oriented behavior in their daily lives.

On stress

Chapter 5 provides an account of significant stress-induced dopamine activity in the prefrontal and temporal cortices of patients with a psychotic disorder and healthy controls. Positron emission tomography imaging detected intact magnitude and spatial extent of cortical dopaminergic activity under acute stress in patients with chronic psychotic disorders. Moreover, the functional relevance of cortical dopamine in stress modulation was confirmed by showing significant positive associations between stress-induced DA activity and subjective stress experience in both groups of participants.

Large body of literature suggests that early life stress, childhood trauma, can "inoculate" the dopaminergic systems to better endure stress later in life, and that this process might be absent in psychosis due to the noxious effect of both childhood trauma and current stress in this group. **Chapter 6** therefore investigates the effect of childhood trauma on stress-related DA activity in the adulthood in the existing data from chapter 5. In the healthy controls, more severe childhood trauma is associated with more extensive dopamine activity under stress in the medial prefrontal cortex, hinting at a mechanism of adaptive calibration of the prefrontal dopamine signaling of stress. No such relationship was observed in patients with chronic psychosis, however, suggesting a deviation from this adaptive pattern in this group.

Chapter 7 further explores areas of the brain that are involved in the modulation of negative affective experiences such as fear and pain, important constituents of the stress response. The cerebellum has reliably been implicated in learning and memory, and increasingly recognized for its role in fear conditioning. This chapter presents the activation likelihood estimation meta-analysis that pooled all existing functional neuroimaging studies of fear conditioning in healthy participants, and identified four loci of cerebellar activation during the acquisition of fear, warranting further exploration of stress processing in this region.

On stress and reward

Chapter 8 operates on the assumption that in real world, rewards are always encountered against the background of a variable degree of stress, and tests the premise that stress affects the experience of reward. Indeed, both in the laboratory and in the daily-life stress was found to have a negative impact on reward capacity of healthy individuals, with a potential to trigger the severe reward deficits seen in psychosis.

Chapter 9 summarizes the findings of the human normative reactivity to stress and reward, and the deviations, or the lack thereof, in individuals on the spectrum of psychosis. Patients demonstrated largely intact dopaminergic reactivity to stress, and their relatives showed intact reward-related dopamine activity. Only when childhood trauma was considered, and reward deficit was tested behaviorally, meaningful differences between healthy controls and those further on the spectrum of psychosis emerged. Contrastingly, in healthy controls the exposure to positive and negative environmental factors was found to have a decisive impact on reward capacity and behavior. One is thus compelled to wonder whether psychosis might be the results of a vicious cycle of environmental adversities, with the brain at its center doing precisely what it evolved to do - interacting.

Valorization

The primary goal of science is to improve the quality and extend the quantity of the human life. Governments and private corporations fund scientific research because technological advances, effective medical and psychological interventions, and pro-social behavior allow us to be more productive and use up less resources, which ultimately generates greater prosperity.

Few things are better determinants of people's functioning and achievement than the capacity to pursue rewards and cope with stressors. Facilitating reward-oriented behavior while at the same time maximizing resilience to stress in the general population is therefore an attractive endeavor for the private sector as well as the society at large. Moreover, pathological motivational impairments and stress vulnerability are an integral characteristic of several psychiatric disorders, but particularly the psychotic disorder. These deficits have been shown to play a crucial role in alarmingly high unemployment rates (80-90%) insufficient physical activity, limited community functioning and poor self-care among patients with psychosis, thus posing a significant public health concern.

This dissertation explored the healthy and abnormal sensitivity to rewards and stressors, and proposes that the brain's dopaminergic systems play a central role, but are not necessarily the causative factor. Instead, the findings of this dissertation arrive at the conclusion that the environmental adversities first impact on the dopaminergic systems, resulting in loss of robustness against every subsequent exposure to stress and absence of reward, until psychopathology emerges. Naturally, this notion that the environment in interaction with dopamine might precipitate psychosis has far-reaching implications for the clinical practice, future research and industry.

Optimizing person-environment interactions: implications for clinical care

Currently, medications indiscriminately blocking the dopaminergic neurotransmission are the cornerstone intervention for psychotic disorder, often as part of a long-term symptom management program. Only in a framework where each individual patient's environmental demands and incentives are taken into account, personalized psychiatry emerges. One example are mobile health (mHealth) interventions that essentially supplement the person's daily environment, offering insight into the maladaptive patterns and stimulating behavioral change. The research presented in this dissertation suggests that mHealth interventions modifying behavior towards stress reduction and pursuit of meaningful goals are likely to be particularly effective in psychosis.

Furthermore, psychosis is shown to have a strong genetic component, and tends to strike already at an early age. This dissertation demonstrated that in individuals with a familial predisposition to psychosis, optimal reward sensitivity might confer resilience to psychosis. Translated to preventative care, these results indicate that it would be advisable to enrich the environment of children and adolescents at familial risk for psychosis with rewarding experiences (youth camps, after-school programs and team

sports). To determine the effectiveness and feasibility of interventions designed to improve person-environment interactions, however, randomized clinical trials using at-risk populations are warranted.

Optimizing person-environment interactions: implications for the industry

Self-quantification and "biohacking" technology is a booming industry with a broad market worldwide; From hard-charging athletes striving to maximize performance to CEOs seeking to enhance their creativity, to ordinary individuals pursuing weightloss goals, improved stress management and reward capacity are the core outcomes of any behavior modification protocol. The design of wearables and mobile applications is therefore directly informed by advances in understanding of the human stress and reward response. This dissertation demonstrated that in the daily life, positive affect experienced together with physical activity increases the odds of engaging in physical activity in the near future. This finding has profound consequences for any behavior activation protocol: providing rewards contingent upon desirable behavior is likely to stimulate that behavior, and foster the formation of good habits. At the same time, social stress was found to temporarily decrease the ability to enjoy rewarding activities, thus interfering with the propagation of positive behaviors. A tool designed to optimize mental and physical performance should therefore foremost assist in mitigating social stress, while providing frequent positive feedback immediately upon an instance of preferred behavior. The research included in this dissertation can therefore provide efficacy and competitive edge to service and product developers. The exchange of this knowledge has already been initiated with Department of Computer Engineering of the Kwangwoon University in South Korea, specializing in the design and application of next-generation of performance optimization devices.

Optimizing person-environment interactions: implications for research

This dissertation directly contributes to the current scientific debate on the dopamine hypothesis of psychosis, subjecting the purely neurobiological perspective to scientific scrutiny. In particular, it supports the notion that deterioration of dopaminergic neurotransmission and brain function in individuals further on the spectrum of psychosis is not a closed-circuit, intrinsic process, but rather secondary to adverse environmental exposures.

Furthermore, the research studies incorporated in this dissertation often employ unconventional approaches, such as the combination of experimental laboratory measures with momentary assessments in the daily life that provide unique evidence for an emerging phenomenon of stress-related anhedonia. Adopting new methods and challenging established viewpoints arguably generates new questions and thus forges progress, an endeavor that is vital for the field of schizophrenia research in particular

and the scientific community in general. The insights and approaches presented in this dissertation have been made publicly available through publications in open-access journal articles and presentations at scientific conferences worldwide.

Acknowledgements | Poďakovanie

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Curriculum vitae

Zuzana Kasanova was born on October 25th, 1984 in Nitra, Slovakia, where she attended a bilingual Slovak-Spanish high school, and in parallel, was a member of the UKF Nitra women's basketball team. Upon her high school graduation in 2004, she enrolled into a Psychology program at the University of Maryland, Baltimore County on a full athletic scholarship for basketball. Throughout the undergraduate program, she completed a series of internships at the Kennedy-Krieger Institute where she provided behavioral therapy to children with developmental disabilities. She graduated with a bachelor's degree in Psychology in May of 2008, and began working as a research assistant in the Cognitive and Affective Neuroscience Schizophrenia lab of Prof. Jim Gold at the Maryland Psychiatric Research Center. In 2011 she was awarded the High Potential Huygens Scholarship and enrolled into the Research Master of Cognitive and Clinical Neuroscience at the Maastricht University in The Netherlands. As part of this program she completed a 9-month internship at the Department of Psychiatry and Neuropsychology of the Maastricht University in the team of Prof. Myin-Germeys. Zuzana was then awarded the Kootstra predoctoral fellowship that partly funded her full-time doctoral training in the same group, during which she performed research into stress and reward reactivity in psychosis in collaboration with RWTH University Aachen. Along her research activities, she taught bachelor and master-level courses at the Faculty of Health, Medicine and Life Sciences, and at the Faculty of Psychology and Neuroscience of the Maastricht University. In addition, she worked as a psychologist and case manager to patients with psychosis as part of her clinical training in the FACT team of the Mondriaan Zorggroep. In the last year of her PhD trajectory, she also enrolled into the doctoral program in Biomedical Sciences at the KU Leuven as part of a joint PhD degree.

LIST OF PUBLICATIONS

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SUBMITTED WORK

Kasanova Z, Ceccarini J, van Amelsvoort T, Frank M, Booij J, Heinzel A, Rong Y, Winz O et al. Striatal dopaminergic modulation of reward processing in healthy individuals at a genetic risk for psychosis. Schizophr Bull. submitted

Kasanova Z, Sterbova S, Collip D, Myin-Germeys, I. Social stress is associated with reward dysfunction: Converging evidence from the laboratory and the daily-life. Psychological Science. submitted

Batink T, Bakker J, Vaessen T, Kasanova Z, Collip D, Myin-Germeys I et al. The ACT in Daily Life Training: a feasibility study of a mHealth intervention. Journal of Medical Internet Research. under review.

Colombi M, van Heugten C, Rasquin S, Leneart B, Kasanova Z, Ponds, R. Exploring the feasibility and usability of the experience sampling method to unravel the daily lives of patients with acquired brain injury. Neuropsychological rehabilitation. submitted